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EXPERIMENTAL POPULATIONS[1]

EXPERIMENTAL POPULATIONS OF MICROSCOPIC ORGANISMS

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ACCORDING to Pearl (1930), a population may be defined as "an aggregation of individual organisms of the same species, living together in a limited and defined universe." This definition is applicable to most of the experimental studies discussed in this paper. An exception is found in the very interesting studies of Gause (1934), who has worked with mixed populations.

The investigations of populations of microscopic organisms may be divided into two classes: (1) those in which the organisms are able to obtain their nourishment from the medium in the form of dissolved substances, and (2) those in which the organisms seem to require particulate food, usually bacteria, although in some cases algae or protozoans, or both; from one point of view the latter are also mixed populations. Studies of the first type have been made on bacteria, yeasts, flagellates and a few species of ciliates. Most of the studies of the second type have been made on infusions of ciliates. Due to the time limits of this paper the discussion will be concerned principally with results that have been obtained on populations of ciliate protozoans grown on bacteria.

Of primary concern in these investigations are the relationships between the density of the population and the

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environment. The use of bacteria or other organisms as food in population studies of ciliates makes the analysis of such populations more difficult than the analysis of populations growing in the absence of other organisms. In spite of the difficulties involved we now have a considerable amount of information concerning the effects of different factors on such population growth.

Woodruff (1911) was the first investigator to study carefully this problem. He followed the course of populations of *Paramecium aurelia* for from 16 to 20 days in 2, 5, 20 and 40 drops of hay infusion, which were changed at 24 and 48-hour intervals in different series of experiments. These were compared with similar populations of *P. caudatum*. He found, in both cases, that the fission rate is higher, for the periods and amounts of medium tested, the larger the amounts of available medium. He also found that the rate of division of the 2-drop cultures was greater in the cultures changed every 24 hours than in those changed every 48 hours. And finally, he found that culture media in which paramecia had flourished for 10 days was not as suitable for the growth of other paramecia as similar media which had stood for 10 days with no paramecia growing in it.

From these observations Woodruff concluded that the reproductive rate of *P. aurelia* and of *P. caudatum* is influenced by the volume of culture medium, and that in the volumes tested, the larger the volume of medium the more rapid is the rate of reproduction. He decided that the differences were due to toxic excretory substances, produced by the organisms themselves, which are more effective in small volumes of culture medium. Woodruff cross-inoculated all the cultures used and because of this believed that the bacteria, as agents in causing the differences in the division rate of *Paramecium*, were eliminated. Due to other data which will be discussed later in the paper, it should be noted here that the kinds of bacteria present in the cultures, and the numbers of bacteria present were not known in these studies.

This problem remained much as Woodruff left it until Robertson (1921a and 1921b) described opposite results with two infusorians. He found that when two Infusoria (*Enchelys farcimen* or *Colpoda cucullus*) are introduced into a small amount of fresh culture medium, the early rate of reproduction is more than double that of a single individual of the same species in the same volume of culture medium. Robertson designated this increased rate of reproduction as the "allelocatalytic effect." Allee (1931, p. 166) gives the following condensed statement of Robertson's hypothesis: "During nuclear division each nucleus retains the charge of autocatalyst with which it was provided, and adds to it during the course of nuclear synthesis. At each division the autocatalyst is shared between the nuclear substance and the surrounding medium in a proportion determined by its relative solubility and by its affinity for chemical substances within the nucleus. The mutually accelerated or allelocatalytic effect of contiguous cells is due to each cell's losing less of the autocatalyst to the medium because of the presence of the other." In his later papers Robertson stated that this effect is more marked when the organisms have been freed from contamination with the old culture medium by repeated washings. In his last paper he repeated that all his conclusions concerning allelocatalysis in Infusoria were valid except that they might apply to the food organisms.

Following the publication of Robertson's results, numerous investigators made similar studies, using different organisms.

Cutler and Crump (1923a and 1923b), using *Colpidium colpoda,* were unable to obtain the effect described by Robertson. In a like manner Greenleaf (1926), using *Paramecium aurelia, P. caudatum, Pleurotricha lanceolata* and *Stylonychia pustulata;* Calkins (1926), using *Uroleptus mobilis;* Myers (1927), using *P. caudatum;* Grimwald (1928), using *C. colpoda;* and Di Tomo (1932), using *P. caudatum,* report results which are contrary to

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the theory of allelocatalysis. Jahn (1929), working with mass cultures of *Euglena* sp., studied the relation between density of population and the growth rate and found no evidence of any allelocatalytic effect. Beers (1933), like Jahn, eliminated the bacterial food supply in his studies on *Didinium nasutum* and *Stylonychia pustulata.* He used salt solutions instead of hay infusions and fed *Didinium* on washed paramecia and *Stylonychia* on measured amounts of the phytomonad *Chlamydomonas.* Using different numbers of each test organism in different volumes of medium, he obtained no indication of the Robertson effect.

On the other hand, Yocom (1928) described results with *Oxytricha* sp. which he interpreted as allelocatalysis. Yocom found that the division rate of 1-animal cultures in 4 drops of medium was 10 per cent. greater at the end of 24 hours than 1-animal cultures in 10 drops of medium. Peterson (1929), working with *Paramecium caudatum,* obtained results that were both in favor of and opposed to the Robertson effect. When she used 5/24 cc of hay medium or less, the 2-animal cultures reproduced no faster than 1-animal cultures in 24 hours. When she used 20/24 cc of medium, the 2-animal cultures reproduced faster than the 1-animal cultures. Dimitrowa (1932), in an attempt to test the effect of excretion products on the rate of reproduction in *P. caudatum,* reported some results which might be interpreted as favoring the Robertson theory. When paramecia were isolated into 0.5-cc of medium containing several drops of an old culture medium in which 10 to 100 paramecia had lived for 24 hours, the rate of division was decreased. But when paramecia were introduced into a similar volume of medium in which one small drop of a much less contaminated old medium had been added, the paramecia divided more rapidly than those placed in a control culture which had not been so conditioned. Most of the investigations cited above have been carefully reviewed by Allee (1931 and 1934).

The investigations of the writer (Johnson, 1932) on the effects of population density on the rate of reproduction

in the infusorian, *Oxytricha fallax,* were made with the aim of finding out something more about this disputed question. In the first phase of this work the protozoans were grown in ordinary hay infusion with unknown mixed bacteria as the source of their food. Using sister cells from pure line cultures, there was no difference in the reproductive rate when *Oxytricha* was introduced singly or in pairs in one or two drops of bacterized hay infusion for the first 24 hours. Beyond this period the cultures originally containing one animal had a higher rate of division. Five-drop cultures started with two infusorians had a higher rate of reproduction at the end of 24 hours than similar cultures started with one organism. In a similar way the progeny of 2-animal cultures exceeded those of 1-animal cultures in ten drops of medium. Several tests were made, using both volumes, and in each case the results are statistically significant.

When sister cells taken from the same clone were cultured singly in 2, 5, 10, 20 and 40 drops of hay infusion, the mean population from 14 series at the end of 24 hours was 6.3, 5.6, 4.8, 4.1 and 3.5, respectively. The differences between any two populations are significant. These results are shown in Fig. 1.

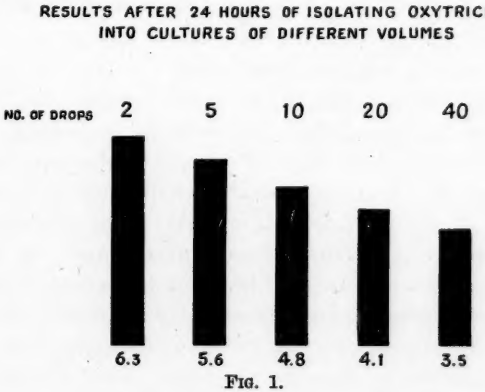

FIG. 1.

Keeping the conditions comparable throughout a given series, changes in the temperature and changes in the

hydrogen-ion concentration did not alter these relationships. Darby (1929 and 1930) suggested that the results described as allelocatalysis might be due to the hydrogen-ion concentration. According to his suggestion, if the medium was not at the optimal H-ion concentration for the species and was weakly buffered, the protozoan might change the H-ion concentration toward the optimum and two protozoans could change it quicker than one in similar volumes of medium. But in this work changes in the H-ion concentration of the medium did not alter the relationships. In these experiments two types of results were obtained, depending, apparently, on the relationship between the volume of medium and the number of protozoans present.

In the second phase of this work an attempt was made to find out whether the food organisms, the bacteria, could be responsible for the results observed. To do this it seemed necessary to control the bacteria, both qualitatively and quantitatively, and to use a reproducible medium. In the investigations referred to above, in only one case (Cutler and Crump, 1924) was the food organism actually known, and the hay infusion used in most of these researches is not a closely reproducible medium. The use of a single kind of bacteria as food for protozoans has been found possible in several instances. (*Cf.* Luck, Sheets and Thomas, 1931). Suspensions of different kinds of bacteria in a balanced salt medium as used by Barker and Taylor (1931) were tried as food for *Oxytricha.* Of the seven kinds tried only two supported continuous growth of *Oxytricha.* The division rate of *Oxytricha* on suspensions of the bacterium, *Pseudomonas fluorescens,* was greater than that obtained in hay infusions. The bacteria used in these tests had been taken from a hay infusion culture of *Oxytricha.* Such results indicate that not all the bacteria present in such cultures are suitable as food for *Oxytricha.*

The density of these bacterial suspensions seemed to affect the division rate of the protozoans. Suspensions made with a standard loopful of bacteria were designated

as X concentration. In these experiments X equals approximately 10^8 per cc. When single *Oxytricha* were introduced into 2 drops of 4X, 2X, X, X/4 and X/10 concentrations of *Pseudomonas fluorescens* the mean results at the end of 24 hours were 3.54, 9.0, 11.4, 5.37 and 2.98, respectively. These relationships are shown in Fig. 2.

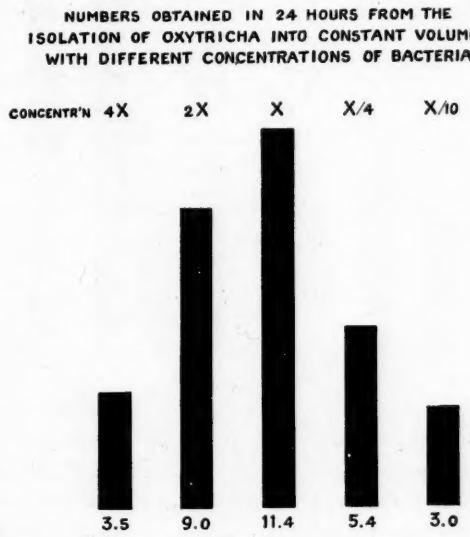

FIG. 2.

These results show that there is an optimum density of bacteria for the maximal reproduction of this protozoan. Barker and Taylor (1931, p. 623) made similar observations in their studies on *Colpoda*. "If too many bacteria surround the protozoa, the latter are adversely affected as shown by a strongly reduced division rate. In an exceedingly dilute suspension of bacteria the protozoa show reduction in size and division rate."

When one and two *Oxytricha* were introduced into 2 drops of X concentration there was no difference in division rate for 24 hours. But in experiments made at the same time and in the same way, using 2X and 4X concentrations, the cultures started with 2 protozoans had re-

produced faster than those started with one. The results from 14 series of experiments are shown in Fig. 3.

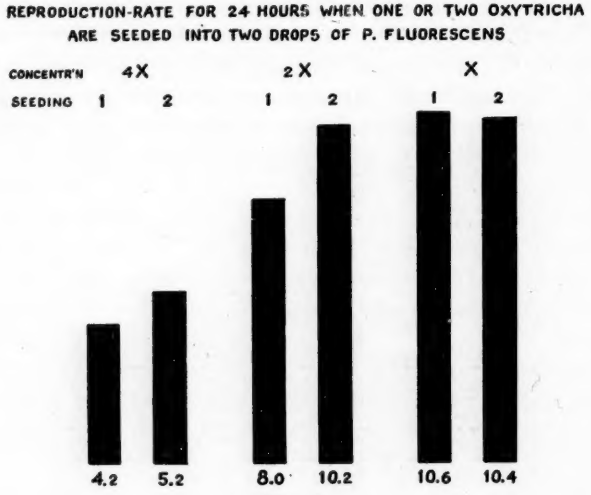

FIG. 3.

The differences with 4X and 2X concentrations are statistically significant. When 5X concentrations were used there was little reproduction, and some of the animals died. The exact nature of this bacterial crowding is not understood at present. Jahn (1934) has suggested that it might be due to excess CO_2 produced by the bacteria. Other experiments showed that in 2 drops of the X concentration there was sufficient food to permit maximal reproduction of *Oxytricha* for 24 hours; the use of 10 drops of the same suspension did not increase reproduction. It was also found that the use of 10 drops of X/10 concentration supported greater growth than 2 drops of the same concentration. Fig. 4 illustrates this relationship.

In many respects the data of Chejfec (1929) support the above observations. He found that the division rate of *Paramecium* depends on the number of bacteria. Table I, taken from Chejfec, illustrates his findings.

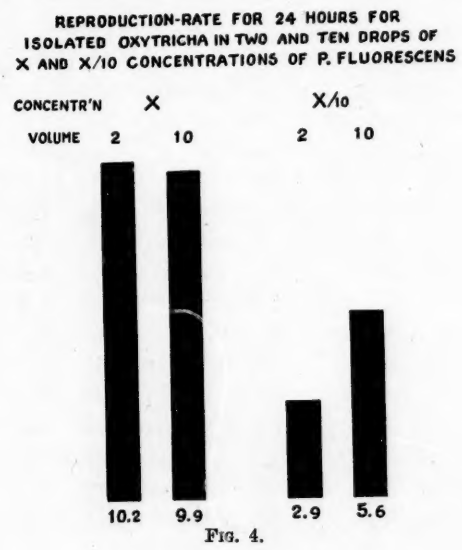

FIG. 4.

TABLE I

INDIVIDUAL NUMBER IN DIFFERENT CONCENTRATIONS OF *Bacillus coli* AFTER 2, 4, AND 6 DAYS (FROM CHEJFEC)

Days	Initial seeding	*Bacillus coli* per cubic millimeter				
		25,000	50,000	75,000	125,000	175,000
2	25	366	323	244	197	127
4	25	583	783	1,665	1,872	2,428
6	25	486	1,045	1,686	3,476	6,680

This work with *Oxytricha* confirms the existence of the phenomenon described by Robertson, but it does not confirm his theory of allelocatalysis. In cultures where the bacterial concentration is supra-optimal it appears that two protozoans are able to reduce the numbers to the optimal density quicker than one, and as a result exhibit a higher reproductive rate. This indicates a beneficial effect of crowding in protozoans when they are in media very dense with bacteria.

When the results using bacterial suspensions are compared with those in which hay infusion was used, it appears that the differences in the hay infusion cultures were also due to differences in the numbers of bacteria. It was found that one drop of hay medium would support the

progeny of one or two *Oxytricha* for 24 hours without any significant difference in reproduction, but at the end of 42 hours the cultures started with one protozoan showed the higher division rate. This seems to be due to a quicker exhaustion of the food supply in the two-animal cultures. When 5-and 10-drop cultures were used, the two-animal cultures had a higher rate of reproduction at the end of 24 hours. These volumes of hay medium will support greater numbers of bacteria than one drop will, and so it seems plausible to suggest that bacterial crowding was occurring in the larger volumes of hay medium. This suggestion is supported by the observation that dense clouds of bacteria existed in these larger volumes after 24 hours.

Similar suggestions have been made by other workers. McPherson, Smith and Banta (1932) found that groups of *Paramecium* have a higher initial rate of fission in "strong" medium than do isolated animals; in "weak" medium they found that the reverse is true. Smith (1932) in studying the effects of strength of culture medium on *Paramecium* found that medium weaker or stronger than his normal medium reduced division. In these experiments the strength of the medium would determine the number of bacteria present.

Studies were made on populations of *Paramecium* reared on *Bacillus subtilis* for a period of several days (Johnson, 1936). Using this kind of bacteria as food for *Paramecium,* it was necessary to use quite dense suspensions to produce the effect of bacterial crowding. Populations of *Paramecium* started with one and five animals in 0.25 cc of both X and 5X concentrations of *B. subtilis* were followed for 7 days. Fig. 5 gives the results of 4 series of experiments.

The highest rate of reproduction for the first day was obtained in the X concentrations with little difference between the singles and the group. After the first day the rate of division was highest in the 5 X concentrations. The greater reproduction of the groups in the 5 X concentrations during the second and third days is like the

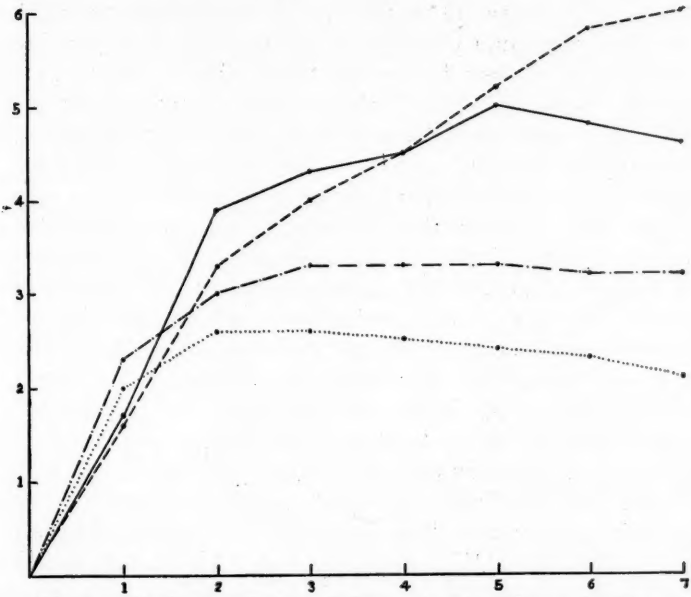

FIG. 5. Showing populations of *Paramecium* in different densities of bacteria. Numbers on the ordinate denote the number of fissions, while those on the abscissa denote the number of days. (————) groups in 5× concentration, (- - - - - - - -) singles in 5× concentration, (. . . . . .) groups in × concentration, (——·——) singles in × concentration.

situation found in studies on *Oxytricha* and attributed to bacterial crowding. All the cultures had reached their maximum populations by the end of the seventh day except the singles in 5 X concentration. They reached their maxima by the ninth day. In no case did the 1-animal cultures in either concentration of bacteria reach as high maximum populations as the 5-animal cultures reached. In Fig. 5, five 1-animal cultures are compared with one 5-animal culture in each case. Actually the highest maximum reached by any 1-animal culture in 5 X concentration was 96 as compared with 162 in the 5-animal culture. This relationship also existed in the X concentration. When these results are compared with those of Myers (1927), who used hay medium, certain differences are

noted. Myers found that 1- and 2-animal cultures in 0.2 cc of hay medium produced the same maximum population, while the 4- and 8-animal cultures had a lower maximum. The differences were attributed to the greater accumulation of waste products in the early stages of the 4- and 8-animal cultures. In this work the relationships are essentially the reverse.

In these experiments the facts that the highest fission rates occurred during the first two days, that there were some bacteria even in the X concentration cultures at the end of seven days and that the rate of reproduction of the paramecia, even in the single-animal cultures, did not increase proportionately after the second day, suggest that some change in the bacteria might be responsible for the differences. A few workers have been able to grow protozoans on dead bacteria (Oehler, 1919; E. and M. Chatton, 1923; and Johnson, 1936); but other workers (Luck, Sheets and Thomas, 1931; Phelps, 1934; and Hetherington, 1934) have found that dead bacteria would not support growth of different protozoans. In these experiments it seems that changes in the bacteria (possibly spore formation, starvation and death) in the non-nutritive medium over a period of days might make them unsatisfactory as food for *Paramecium*. With the test organism, the kind of food used, the temperature and the hydrogen-ion concentration controlled and with the nature of the medium known and reproducible, it is shown that numbers of bacteria very definitely affect the protozoan population. Very little is shown in this work on the rôle of waste products as a limiting factor in the population growth. The results given in Fig. 5 might be interpreted as due to waste products, but are they entirely? If the waste products are a factor here, to what extent are they a factor and in what relationship to the food supply? Nearly every discussion of protozoan population studies refers to the effects of waste products, but we need to know the nature of these effects in relation to the food supply. Phelps (1936) obtained populations

of *Glaucoma pyriformis* grown in pure culture on yeast extract as dense as 500,000 per cc. He states that concentrations of animals more than 1,000 times greater than those observed in other investigations were obtained before the effects of excretory products on division rate were noticed.

In some recent work (unpublished) the writer has studied populations of *Paramecium* grown in old culture medium. In this work 80 animals were isolated into 5 cc of a suspension of *Pseudomonas* sp. in salt solution. At the end of 24 hours the animals were removed and counted, fresh bacteria were added to the medium and 80 paramecia were introduced into the medium again. This procedure was repeated each day. The average fission rates for 16 days were 1.74, 1.13, 1.48, 1.45, 1.34, 1.47, 1.53, 1.29, 1.31, 1.41, 1.00, 1.06, .37, .41, .23 and .25. There was no consistent difference between the results obtained in these cultures and those in controls run at the same time where the medium was fresh each day until after the tenth day. At this time the experimental animals were noticeably smaller and less active than the controls. This difference seems to be due to accumulated waste products, produced, perhaps, both by the protozoans and the associated bacteria. In these experiments the ratio of volume of medium to each *Paramecium* was one drop for each animal (16 drops equals 1 cc) at the beginning of each 24-hour period. By the end of the tenth day over 2,600 paramecia had lived in the 5 cc of medium. The relationships of volume of medium to the protozoans here are similar to those of other investigations, where the limiting effects of waste products were described as occurring in 24 to 48 hours. These results suggest that in such investigations in which the food of the protozoans was not under control a depletion of the food supply might have been more of a limiting factor for continued growth than the accumulation of waste products. More work needs to be done on this point.

All the experiments discussed up to this point have been concerned with populations of a single kind of Protozoa.

In nature populations of microscopic organisms usually exist as mixed populations. In recent years Gause (*cf.* Gause, 1934) has introduced the study of mixed populations of micro-organisms as a means of obtaining experimentally further understanding of the struggle for existence. His studies have included mixed populations of yeasts, mixed populations of Protozoa feeding on the same bacterial food supply and populations of the predator-prey type, with *Didinium* feeding on *Paramecium*.

Gause's work may be illustrated by describing the results obtained in mixed populations of *P. caudatum* and *P. aurelia* where the two species were competing for the same supply of bacteria. The protozoans were grown in 5 cc cultures. The medium consisted of a single kind of bacteria suspended in balanced salt solution. At the end of each 24 hours the cultures were centrifuged, the old medium was drawn off and fresh medium was added. Gause states that by this procedure he eliminated the factor of waste products. Populations of each species grown separately were studied first. In that part of the work he found that for a while there is a period of regular increase in the population. At the end of this stage the possibility of further growth is exhausted. Following this, due to the replenishing of the food each day, a fairly constant population is maintained. He found that *P. aurelia* has a higher rate of reproduction than *P. caudatum*. However, when he calculated the volumes of the two organisms he found that the growth by volume was about the same in each case. When he grew the two species together he found that *P. aurelia* eventually crowded out *P. caudatum*. This is apparently due to the advantage which *P. aurelia* has in having a higher reproductive rate.

In this work Gause has been concerned with the mathematical analysis of the struggle for existence. In his mixed populations of yeasts the experimental data actually fit the theoretical equations describing the various phases of the struggle for existence. In the case of

mixed populations of infusorians the situation is not so simple. This line of attack on population studies is a valuable one. It seems, however, that the further development of this method of attack must be accompanied, if not preceded, by additional experimental analyses of the factors which affect such populations.

In this report some emphasis has been placed on the importance of controlling the food supply, both the nature of the food and the amounts of it, in population studies of micro-organisms. This emphasis, however, does not imply that food is the only important factor in such studies. Along with the food supply we need to consider the nature of the medium, both as to salt content and hydrogen-ion concentration, the temperature, the nature of the experimental organisms and other factors which may be shown to affect such populations. Only through a better understanding of the interrelationship of all the factors concerned and by greater and greater precision in the control of these factors can we obtain results which are reproducible and predictable.

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EXPERIMENTAL STUDIES OF INSECT POPULATIONS[1]

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I

A NUMBER of investigations have been made during the last decade which properly can be classified under the heading, "Experimental Studies of Insect Populations." In fact, these reports are becoming so numerous that it is difficult to exclude and choose material calculated to represent the field for the period of a half-hour talk. Probably the best way to do this is to organize the data around a specific theoretical conception and show how the results of a few selected investigations bear on this problem. Many approaches could be explored with profit. For example, the effects of numbers on protection from toxic substances might be stressed. This is an emphasis germane to the interests of the economic entomologists, since it has application to the control of insect pests. The results of several independent studies suggest that groups of insects survive the effects of various environmental poisons better than do isolated individuals. Another instructive and biologically significant aspect of the insect population problem is the relation between crowding and morphological changes. This can be illustrated briefly by studies made on aphids and locusts. It has been shown that the former produce wings when distinctive conditions of density are realized and that a similar stimulus is probably responsible for the formation of structural phases in certain locusts. These phases involve such characteristics as the coloration of the nymphs, the form of the pronotum, the size of the body and the relative length of the imaginal wings. (For a detailed summary of these and other studies see Allée, 1931; 1934.)

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It is obvious, however, from a survey of investigations dealing with these matters that one of the factors which has received a great deal of attention by students of insect population is density. This is so, not simply because the effect of density on populations is something which frequently lends itself to experimental analysis, but because population students see in density a problem eminently biological, since it is a result of the *number* of organisms themselves, and important, since it is invariably one of the most influential factors guiding the course of the population. This general position can be summarized succinctly in the words of Professor Pearl, who has said:

> In general there can be no question that this whole matter of influence of density of population, in all senses, upon biological phenomena, deserves a great deal more investigation than it has had. The indications all are that it is one of the most significant elements in the biological, as distinguished from the physical, environment of organisms. (1930: p. 145.)

However, these are facts more or less known to every one and probably deducible on *a priori* grounds alone. The main thesis of this paper takes off from this background and asks the questions: What are some of the influences of density on populations of insects and in cases where it is known, how does density bring about its described effect? The latter point is, I think, an important one and will be the primary emphasis behind my remarks. Too frequently, when it has been shown experimentally that a certain effect is associated with density the conclusion is drawn *post hoc, ergo propter hoc* that it is the number of organisms acting upon each other in a behavioristic or even "psychological" fashion which is solely responsible for the observed result. In some cases, as I hope to show, this may be true: in other cases, density may be broken up into different units and shown to be acting through such secondary effects as the reduction of the available food supply, the charging of the medium with waste-products and other general modifications of the environment. Undoubtedly, in all natural populations where the actual number of organisms is not experi-

mentally divorced from such alterations of the environment, both types of effects are operative, though not necessarily to an equal degree.

II

The experiments of Pearl and collaborators illustrate some of these points. Using *Drosophila melanogaster* these investigators (Pearl and Parker, 1922) set up initial populations of flies at concentrations ranging from one pair per half-pint bottle to 50 pairs per half-pint bottle and counted the number of progeny produced by the different densities. They were able to show that as the population density increased the number of offspring per bottle decreased. In other words, productivity stood in inverse ratio to population density. The authors made no attempt to explain this descriptive result at the time, simply pointing out that the phenomenon was one characteristic of many types of populations and that the data conformed to a generalization made by Farr, in the middle of the last century, with regard to the deleterious influence of crowding on the death-rate of man.

In a later paper, Pearl (1932) designed experiments to answer the following two questions: first, what physiological process or processes which would explain the results just described is influenced by density, and, second, what factor or factors, dependent upon density, influence this process? In considering the first point it was reasoned that in short-lived populations where mortality was not important the rate of reproduction must be the factor varying between the different densities. In other words, the flies would have to reproduce faster at low densities than at high to explain the observed results. This differential in reproduction could be due to an alteration of fecundity—the actual number of eggs produced; to an alteration of fertility—the ability of the eggs to hatch into larvae or to an alteration of both. Experiments were started in which the initial densities varied in geometric series from one to 128 pairs of *Drosophila* per bottle. The eggs were collected at regular intervals from

each container and counted. It was shown that the fecundity was greatly influenced by the number of imago flies in the bottle, since the oviposition, expressed as a rate per female per day, fell off rapidly as the density increased. No important relations between fertility and density were reported.

From this particular analysis two facts emerge: first, the description of a specific relationship between population density and population growth, and, second, the demonstration that this relationship is due to a differential alteration of the rate of oviposition. An analysis of the factors actually influencing the fecundity logically follows as the next step. To answer this question Pearl set up experiments in which two things were varied: the air volume in the bottles above the agar medium and the surface area of the medium itsef. Each of these two types of containers were then run with varying densities of flies and the egg production assayed. It was found that, while the extent of the air space above the culture medium had no significant effect on oviposition, the extent of crowding of the flies on the agar surface was highly important in altering their fecundity. To put it differently, the real density effect occurs when the flies are crowded together on this surface. By making a careful series of observations on the behavior of crowded and isolated flies Pearl reached the conclusion that *Drosophila* will not oviposit if they are in contact with or disturbed by other flies, and that individual flies do not obtain as much food under such conditions even though there is an ample supply of food present for their consumption. This was called the "interference" or "collision" effect, and it was shown that the probability of collisions between *Drosophila* on the agar surface increased with the density and that the pattern of the collisions was somewhat analogous to the random movement of molecules in a limited universe.

The importance of this investigation lies in the fact that it not only describes an actual density effect and shows that fecundity is the principal physiological variable involved, but that it links the entire phenomenon to two

essential *behavior* mechanism—the inability of *Drosophila* to oviposit and feed adequately when disturbed. These data can be cited as evidence for the earlier point that population density sometimes acts by altering the behavior of its components. For the sake of thoroughness, it should be emphasized that Pearl does not conclude that these are the only factors affecting the fecundity of *Drosophila.*

Another investigation which has resolved a density effect into a behavior mechanism can be illustrated by the work of Chapman (1928) on the flour beetle, *Tribolium confusum.* This beetle spends its entire life-cycle in flour, which can be screened with the eggs, larvae, pupae and imagoes collected for counting. Chapman was interested primarily in finding out if all cultures of *Tribolium* would reach a constant equilibrium point of maximal growth, measured in terms of individuals per gram of flour, regardless of the initial density of the population and the size of the environment. To answer this question he set up two types of experiments. In the first group, a series of flour environments with volumes varying geometrically from 4 to 128 grams of flour were seeded with paired beetles varying from one pair in the 4 gram culture to 32 pairs in the 128 gram culture. In the second group, all the cultures contained 32 grams of flour, but the initial beetle densities ranged from one to 32 pairs. Censuses were taken of the eggs, larvae, pupae and imagoes at intervals. Chapman found that all these populations, irrespective of their initial condition, eventually reached a maximum equilibrium point of approximately 43 beetles per gram of flour which was statistically similar for the entire series. It is interesting to note in passing that these data on the growth of *Tribolium* populations have been analyzed by Gause (1931) and shown to be described empirically by the well-known logistic curve.

Chapman explained this equilibrium on the basis of a behavior mechanism of *Tribolium* which varied in its effect on the population with changes in density. It has

been shown both by Chapman and myself that these beetles and the late instar larvae eat their own eggs, and that this cannibalism varies directly with the number of eggs and imagoes present. In other words, when the egg and imago concentration is high in any population the eggs are eaten faster than when it is low. In young, growing populations the adult beetles and larvae are not numerous as they are in older cultures so that the eggs have a better chance of hatching into larval forms. As the population approaches equilibrium more eggs are being produced, since more females are present; but, also, more eggs are being eaten. The actual equilibrium point, according to Chapman, represents a condition where the relation between number of eggs laid and number consumed is fairly constant and is a situation, of course, in which the maximum effective reproduction occurs. In a later paper, Chapman and Whang (1934) analyzed further some of these relationships and showed that during early population growth the eggs, larvae and pupae undergo rhythmic fluctuations in numbers.

The significance of this case like the *Drosophila* one, lies in that it describes a density effect which is dependent primarily upon the cannibalistic behavior of the beetles themselves. In some ways the *Tribolium* situation, as pictured by Chapman, can be accounted for by simpler mechanistic assumptions than those described for the fruit fly by Pearl. The former is based merely on the probability of a beetle finding and eating an egg without any further consequences except that the egg is "taken out of circulation," while the latter, although requiring the same type of assumption for the "collision" effect, operates through more complex behavior responses—egg production and general nutrition. Personally, I do not feet that cannibalism and its results can be regarded as the only dynamic factor of importance in *Tribolium* populations—to do so would be to run contrary to known experimental facts and to the equally well-known view that population problems are not simple problems.

III

In the investigations reviewed thus far the emphasis has been placed on controlling as far as possible any effects the population might have on the immediate environment and varying only the number of animals. These investigations have yielded certain generalizations about the nature of density. In an attempt to get at the other aspect of the problem, namely, the way in which density modifies its environment and the relation of this modification to the population as a whole, the effect of homotypically conditioned (*i.e.*, self-contaminated) flour upon *Tribolium* cultures is being analyzed. "Conditioned flour" represents a medium which is produced by the beetles in the normal course of their existence and is obtained, for experimental purposes, by using flour which has been inhabited by dense populations of *Tribolium*. It is, therefore, an inevitable product of population activity which owes its existence entirely to the cumulative effects of the breeding and general behavior of the beetles themselves. More specifically, conditioned flour differs from fresh or unconditioned flour in that the beetles inhabiting it have reduced its nutritive value through constant feeding and have added certain by-products, such as excretory and metabolic wastes, not present in the original medium. Actual chemical analyses of conditioned flour are not available at the moment, so that it is not possible to attribute the influences of conditioning on *Tribolium* to anything more specific than the flour itself.

The time is too short to permit any detailed discussion of the experimental results describing the various effects of conditioned flour on *Tribolium*. This has been attempted elsewhere (Park and Woollcott, 1937) in a recent, critical review of the subject which may be consulted for further details. For the purposes of this paper it must suffice to state categorically that the relation of conditioned flour to the cannibalism, fecundity, fertility and metamorphosis of *Tribolium* has been analyzed. With the exception of cannibalism, which is reduced, beetles

living in conditioned flour have their fecundity drastically lowered; their egg fertility not importantly affected, and the duration and mortality of their metamorphosis increased when compared with fresh flour controls. Conditioned flour was also "diluted" or graded with fresh flour to form a number of distinct environments ranging from a heavily conditioned to a lightly conditioned medium. The effects of these differentially conditioned flours on fecundity were assayed and it was shown that, in general, oviposition varies directly with the fresh flour content of the culture.

However, the real interest of this work lies, not so much in the fact that certain physiological relationships between *Tribolium* and conditioned flour have been demonstrated, as in the notion that some of these relationships, which are associated with density, are of importance in shaping the course of *Tribolium* populations. The original emphasis behind the study of the conditioned flour problem grew out of the following two facts: first, that the *rate* at which the medium is conditioned varies directly with the population density, and, second, that conditioning goes hand in hand with population decline. The first point is essential to this discussion in that it emphasizes the association between conditioning and population density. The second point raises the valid question: Is conditioning merely a result of beetle activity and of no significance in the population dynamics, or, is it actually a factor which contributes to the decline of *Tribolium* cultures? This question could not be answered on *a priori* grounds. It was necessary to test experimentally certain effects of conditioned flour on *Tribolium*. Such experimentation has unearthed, in part, the material summarized earlier. The immediate task is to sort these facts and point out which ones, if any, are important in explaining the decline of *Tribolium* populations and which are merely physiological effects not directly related to population growth.

It appears obvious that environmental conditioning is not influencing population decline through the medium of cannibalism. Even though cannibalism is definitely af-

fected, that is, the beetles consume fewer eggs if they are living in conditioned flour than they do in fresh flour, it selects against population decline, since more eggs escape being eaten and thereby have a better chance of hatching into larvae. The relation of conditioning to egg fertility also is not important from the population viewpoint, since it has been impossible to demonstrate experimentally that the viability of the eggs is consistently greater in one type of medium than in another.

On the basis of the experimental knowledge available it seems that the effects of conditioning on fecundity, as well as on larval metamorphosis, are highly important. It is not my purpose to suggest that these are the only factors involved or that conditioned flour is the sole cause of population decline. The data are too limited to support such a view, and other possibilities suggest themselves. It does seem inescapable, however, that the drastic inhibition of fecundity and the increase in larval mortality induced by conditioned flour are real and important forces contributing to the aging of *Tribolium* populations. The experiments with differentially conditioned flours, that is, media made up of varying proportions of fresh and heavily conditioned elements, have added the points, first, that the effect of conditioning on fecundity becomes more drastic as the flour becomes more conditioned, and, second, that even a slight amount of conditioning is detrimental to the oviposition rate. The latter point suggests that *Tribolium* cultures are rarely in an optimal condition in respect of their fecundity, since the flour is usually at least partially conditioned. It is possible, however, that other factors, for example, the large numbers of eggs relative to the few adult beetles and the subsequent reduction of opportunities for cannibalism, compensate for this during the earlier stages of population growth. This would keep conditioning, working through fecundity, from being an important influence until later. It is also possible, although there exist no supporting data at present, that conditioned flour in certain optimal concentrations may have

a stimulating effect upon the *Tribolium* which would be of importance in their population dynamics.

This general phase of the population problem has been developed recently by Professor Allee (1931, 1934), who has shown that for many animals the optimal density for any given response is greater than the minimal and smaller than the maximal density. In other words, in certain populations under certain conditions "undercrowding" is as deleterious as "overcrowding." It is becoming more and more apparent that this type of density effect is as much to be looked for in both experimental and natural populations as is the more frequent inhibiting influence of high density. Examples of the latter have been used primarily in this report simply because they illustrate the points made. Had the emphasis been directed primarily towards a description of the way in which density alters population *trends of growth* both would have been given equal weight. It should be mentioned in passing that there are several studies of insect populations where the "Allee-type" of effect has been demonstrated. The mortality of *Drosophila,* the initial productivity of *Tribolium* and *Calandra* and the copulation frequency of *Sitophilus* are all cases in point.

In summarizing the conditioned flour experiments with *Tribolium* the following two important points stand out: first, that an environmental modification (*viz.,* conditioning) has been described which is a result of the activity of the population itself, and, second, that this modification, like the behavior interactions between individual beetles, is also a definite part of the general density picture and plays a real rôle in guiding the course of *Tribolium* populations.

IV

In a recent paper analyzing some effects of density on populations of the grain weevil *Sitophilus,* MacLagan and Dunn (1935) have presented evidence showing that both behavior and environmental factors are important in influencing the effective reproduction of this form. In

ovipositing, the female weevil lays a single egg in a small cavity which she excavates in a grain of wheat. For populations which are not crowded, that is, where there are many grains for each weevil, the female oviposits only in the hairy apex of the grain and usually avoids damaged seeds. In dense cultures, oviposition occurs indiscriminately over the entire surface of practically all the grains. The authors showed, as might be anticipated, that as crowding increased more eggs were laid per grain, since there were more beetles, but that the rate of egg production per individual female decreased. An interesting relation between the maximum fecundity and the availability of wheat grains grew out of these observations, for it was shown that the female weevils would not oviposit at a maximum rate unless many more grains were present in the environment than were actually used. The authors state this relationship as follows:

> This surprising result indicates that the female *Sitophilus* will not lay this number of eggs unless the number of grains available for oviposition is at least eleven times that actually utilized. Any reduction in this number of grains is accompanied by a reduction in the number of eggs laid per female, despite the fact that she is utilizing only a small proportion of the number of available grains. (p. 133.)

In addition to these effects, which can be designated, somewhat arbitrarily perhaps, as environmental aspects of density, the authors also describe behavior interactions between individual weevils which are important in altering general reproduction. For example, in considering the decreased fecundity of *Sitophilus* with increasing density their explanation is markedly reminiscent of the *Drosophila* situation. Here, they consider the "collision" or increased contacts between weevils in crowded cultures a fundamental factor and remark that, "Although the primary effect is mechanical, it operates organically through the reduction of times available for feeding, ovipositing and resting; thereby causing adverse effects upon the physiological processes of reproduction" (p. 136).

A brief example of the effectiveness and possibilities of the experimental population technique as applied to parasitology can be seen from the recent papers of Salt

(especially, 1936). This investigator used as the host a moth (*Sitotroga*) and as the parasite a chalcid hymenopteron (*Trichogramma*), which lays its eggs within the eggs of the host. He was interested, among other things, in studying the effect of varying the density of the parasites in a given population upon a constant number of hosts. To do this, chalcid flies ranging in numbers from none to 1, 5, 10, 25 and 50 were introduced into cultures containing 100 moths. The results of this experiment can be summarized, in part, as follows: As the density of parasites attacking a fixed number of hosts is increased, (1) the number of hosts that escape decreases; (2) the number of hosts that die increases; (3) the total number of parasite progeny reaches a maximum and then decreases, and, (4) even at extremely high densities of chalcid flies some hosts escape being parasitized. Data like these suggest an important and profitable approach to parasitological problems as well as making a distinct contribution to the field of experimental populations.

In closing this paper it should be emphasized that it has not been my intention to discuss all the important studies contributing to an understanding of the dynamics of insect populations. Many investigations, omitted because of lack of time, would elaborate this report. The ecologists, the economic entomologists, the demographers and the parasitologists, as well as the physiologists, whose emphasis I have tried to develop in this paper, are all turning out material which has much bearing on the general problem.

It should not be gathered from these statements, however, that the field is one which is nicely delineated, well understood or thoroughly explored. This would be a far too optimistic and rodomontade position. There remains a crying need for the development of new techniques and new investigations applied both to such old stand-bys as *Drosophila* and *Tribolium*, as well as to other organisms. Many of the problems yet to be attacked seem, on the face of it, relatively simple. Others appear more complex. For example, few precise data really exist which merely

describe in quantitative terms the growth, maintenance, oscillation and decline of insect populations. It is true that the logistic curve has aided in generalizing about some of these phenomena, but experimental descriptions are still deplorably few and much to be desired. Little is actually known about the equilibrium state of insect populations—whether rhythmic and periodic oscillations, as predicted by the mathematician on a stochastic basis, actually occur and, if so, what mechanisms bring them about. The "mixed-population" or inter-species competition problem, so important in the thinking of theoretical biologists and in the observations of thoughtful field naturalists, has hardly been touched experimentally by students of insect populations. The entire density question, both in its descriptive and dynamic aspects, awaits further prosecution. Such are but a few of the frontiers where energies could be concentrated. It is to be hoped that these and other problems will yield to competent attack in the not-too-distant future, for in their solution lies a closer understanding of the more complex populations which exist in Nature.

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POPULATION DENSITY AS RELATED TO SEX AND TO EVOLUTION IN CLADOCERA

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AMONG the interesting phenomena associated with density of population or aggregation among animals are occasionally to be found profound changes in the sex ratio. I shall endeavor to examine a few such cases in Cladocera and attempt to point out their significance as related both to subsequent populations of the animals concerned and to the evolution of new types.

The male sex is essential to the propagation and continuation of most kinds of organisms. But inasmuch as the primary limiting factor in the productivity of most organisms is the number of eggs which the females of a race make ready for fertilization and a small minority of males can in most species effectively fertilize these eggs, equality in numbers of the two sexes in many animal forms seems biologically unnecessary. Such being the case, it may be worth while to examine certain cases in which males are in the minority, or appear in the population only occasionally, and to surmise concerning the biological meaning and evolutionary significance of this tendency.

First let me emphasize the commonplace fact that male individuals can not give rise unaided to new individuals, except in such rare cases as adventitious shoots in the male of dioecious plants and in budding in unisexual hydra. On the other hand, females in a small minority of organisms, yet in the aggregate in a good many species and races, not only produce eggs which develop without fertilization but parthenogenetically produce vast numbers of uniparental offspring. Such types are often notoriously successful, *e.g.*, the aphids or plant lice, rotifers, certain saprophytic and gall-forming insects, the ubiquitous dandelion and the various daphniae or water

fleas. Under the usual favorable environmental conditions with these organisms males are useless, mere "wasters"; and under these conditions males ordinarily do not occur.

But in most races among animals in which parthenogenesis is the common method of reproduction, sexual reproduction has an essential part in the annual life cycle; and when the appropriate environmental conditions supervene males appear and function in the fertilization of sexual eggs, which eggs possess the special qualities necessary to carry the race over a period of drought, cold or other contingency. The conditions which in certain species of Cladocera call forth males (and the sexual eggs), as needed in the economy of nature, will be discussed with particular reference to the effects of aggregation or population density upon sex ratios.

Cladocera, including species of the common Daphnia, Simocephalus, Moina, etc., live primarily in lakes, ponds and temporary smaller bodies of fresh water the world over. They reproduce ordinarily by diploid parthenogenesis and in general males and sexual eggs are absent from the population. However, save for the exceptional pan-parthenogenetic races, these animals are usually dependent upon the sexually-produced winter egg or dauerei to carry the race over drought or winter when temporary habitats become dried up or completely frozen. The production of the then necessary males and of the haploid sexual eggs is associated with a dense population or with aggregation.

Differences in density of population are associated with differences in sex ratios in a variety of animals other than Cladocera. For example, in a family of parasitic nematode worms, the Mermidae, a grasshopper or other host insect may harbor from one to twenty or more of the parasites (Cobb, Steiner and Christie, 1927). If the parasitism is initiated by the host's taking in with its food a single or three or four eggs of the parasite, these will develop into females—apparently without exception.

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If, on the other hand, eight to twelve mermids develop in a single host some will be females and others males. While if twenty or more develop in a host all are males. A similar situation, a certain degree of crowding, apparently is responsible for male production among a population of ordinarily all-female Miastor, a fly normally inhabiting decayed logs (Harris, 1923). Perhaps conditions related to population density are factors in male production in certain aphids and rotifers, although the situation is less clear in those cases.

The causative factors in male-production in Cladocerans have been the source of much speculation and experimentation. In general in nature and in favorable cultures in the laboratory all daphnians are females. The infrequent and often seemingly sporadic appearance of males and sexual eggs has been ascribed to an innate cycle (Weismann, 1876 *ff.*) which calls forth males and sexual reproduction and thus, according to the theorists who supported this hypothesis, absolves the race from the deterioration resultant from continued parthenogenesis. Later workers (Woltereck, 1911) found this theory inadequate and the, still revered, innate cycle subject to modification by environmental influences; they reported that environmental factors may hasten or delay the appearance of males and sexual eggs. In 1914 Agar (1914) and myself (1914) working independently came to the conclusion that males and sexual reproduction were not essential to the continued well-being of clones of Cladocera and that an innate sexual cycle, at least as applied to the Cladocera races we studied, was non-existent. Agar had reared 47 parthenogenetic generations of *Simocephalus vetulus* and I had reared clones of Daphnia parthenogenetically for from 95 to 100 parthenogenetic generations. In both cases the animals continued as vigorous as ever. Since that time I have had lines of three species of Simocephalus, two species and several varieties of Daphnia, three species of Moina and other Cladocerans from geographically and environmentally different origins which have continued solely by parthenogenesis for

hundreds of generations without loss of vitality or any of the symptoms of degeneration, expected by earlier workers, as a result of continued parthenogenesis. It seems sufficiently demonstrated that the theory of an innate sexual cycle is unwarranted and that, in certain races at least, parthenogenesis may continue indefinitely (Banta, 1925). If male production is not governed by internal factors then it is obvious that external influences must cause the occurrence of males.

In our earlier laboratory experience we found males very rarely and when at all only in older bottles in which the animals had become more or less crowded. When Dr. L. A. Brown and I (Banta and Brown, 1923; Brown and Banta, 1935) came to attempt to control the production of males in Cladocera it seemed logical to bring about dense populations experimentally and thus attempt the production of males. The expedient was at once successful and successful beyond our fondest expectations. Clutch sisters of newly released young were distributed to bottles of culture medium, one per bottle as uncrowded controls and ten per bottle as tests. Uncrowded controls only about once in a thousand times produced males. But the crowded mothers, while variable in the percentage of male young which they produced, on the average gave rise to about 42 per cent. males. Juggle the experimental set-ups as you will (with certain reservations) yet under approximately optimum food conditions, whether reared with a single mother in a vial containing 6 to 8 cc of culture medium or 10 mothers in about 75 cc of culture medium, approximately 40 per cent. of male young will appear in the first clutches of parthenogenetic young. Under normally good culture conditions without such crowding or limitation of volume, medium males will ordinarily not be found.

Further, sisters of controls which produce only female young will, if kept during the proper period in a bottle from which a crowded population of Cladocera has just been removed, produce the normal percentage of male young (Banta and Brown, 1929b). The result is the

same if such females are reared with other small animals as "crowders"—planaria, snails, isopods, other species of Cladocera, etc. (Banta and Brown, 1929a). Hence it seems clear that male production in *Moina macrocopa* and in at least several other species of Cladocera is ordinarily associated with density of population.

Observations on sister Moina, some reared uncrowded and others crowded, show that the uncrowded sisters grow more rapidly and lay eggs and produce young sooner than their crowded sisters (Banta and Brown, 1929d). The former produce only female young; the latter roughly 40 per cent. male offspring. Thus male production is associated with a slower development of the mother and delayed production of young, *i.e.*, with a retardation of the constructive results of metabolism. We have not measured the rate of metabolism of these animals; but it seems fairly safe to assume that a lower rate of metabolism is an essential factor in controlling male production in Moina.

Other influences which lower the rate of metabolism were utilized in further experiments. Lower temperature (Brown and Banta, 1932), other conditions remaining the same, was found to promote male production, though the delay in development and in the production of young was disproportionately large in relation to the percentage of males produced. Chloretone, some of the urethanes and cyanide were used in treatments intended to avoid as far as possible the toxic effects of these substances and yet produce some retardation in development (Banta and Brown, 1930). Sufficient positive results were obtained to warrant the conclusion that these inhibitory substances likewise in some measure stimulate male production as well as retard rate of development.

Conversely, low concentrations of ethyl alcohol were used (Banta and Brown 1929e). These treatments accelerated rate of development and caused earlier production of young. Crowded bottles, which would have produced liberal percentages of male young, if given the

alcohol treatment produced none or a much lowered percentage of males.

Aeration (Banta and Brown, 1929b) of the medium in crowded bottles by shaking the medium occasionally, by bubbling air through the medium, by rearing the animals in shallow dishes instead of tall bottles or by exposing the animals themselves to the air in a thin film of water for a brief period shortly before the eggs are laid—all these aeration treatments largely, sometimes completely, neutralize the effect of crowding upon the sex of the young. The male percentage is greatly reduced or the young are all females.

No treatments of any sort after the eggs were laid or beginning less than four hours before the eggs were laid showed any effect whatever upon the sex of young produced. On the other hand, treatments begun a few hours before the eggs are laid are usually effective and some treatments begun as late as four hours before the eggs were laid were found to be fully effective. Hence about four hours (at 20° C.) before the eggs are laid may be considered the critical period for the control of sex of Moina (Banta and Brown, 1929c).

Presumably the rate of metabolism of the ovarian egg during this critical period is decisive in determining the sex of the young produced. This interpretation has not been proven and would be most difficult to establish by demonstration, but it seems definitely suggested by some of our experimental evidence and is consistent with all our known facts and observations.

It will be noted that "crowding" and consequent male production may be equally well accomplished by rearing ten Moina in 75 cc of culture medium, by rearing one Moina in 7.5 cc of culture medium or by rearing one or more Moina in a bottle with other aquatic animals. While we have not directly demonstrated it, the causative factor in male production in crowding experiments seems to be the by-products of the Moina themselves or of the other animals involved (which by-products affect

the rate of metabolism—the actual physiological mechanism involved). As already stated, aeration by whatever means employed tends to neutralize the male-inducing effect of crowding. Mothers give normal male production if crowded for only a brief period just before and including the critical period for sex control, or if introduced into a formerly crowded environment (*i.e.*, into a bottle from which the former crowded population has just been removed). If, however, the bottle, formerly heavily crowded, is without population for several hours before the introduction of the test mother the male-inducing effect is lost (Banta and Brown, 1929b). These and other observations have led us to believe that the actual causative factor for male production in crowding experiments lies in volatile, unstable or readily oxidizable metabolic by-products which, as stated above, work through their effects upon rate of metabolism (Banta and Brown, 1929e).

We do not know just what these supposed by-products are. Experiments with the introduction of carbon dioxide gas into uncrowded bottles did not cause male production. Likewise, experiments with urea, uric acid, chicken manure, mammalian urine, etc., gave negative results (Banta and Brown, 1929b). Nevertheless, we incline to believe that by-products of metabolism are the causative factors and that our failure to provide a demonstration of this hypothesis results from incomplete knowledge of the precise nature of these substances and ignorance of the proportions of the various constituent by-products produced by living aquatic animals.

Now let us consider the situation with Cladocera in nature. In our latitude some Cladocera races carry over winter from the previous season as relatively inactive animals living at a very low metabolic level, growing exceedingly slowly and usually showing no ovarian activity until the ice leaves their habitats and the water temperature rises to 12°–14° C. Other Cladocera, including Moina, remain as sexual eggs until the spring temperatures rise, when they hatch and with further rise

in temperature become parthenogenetically active. The generation cycle is short (5 to 7 days at 20° C.); the numbers of young produced per day per mother may be as high as 10 or more; the population, provided nutritional and other conditions are favorable, increases with amazing rapidity; the habitat soon contains the optimum population which it can sustain. Reproductive activity, however, does not cease and over-population results. Scarcity of food quickly becomes apparent in the decreased per-mother yield of parthenogenetic young.

We have found in the laboratory that sexual eggs (only one developing at a time in each of the two ovaries) are produced under conditions of acute scarcity of food (Wood, 1928; Berg, 1931; Wood and Banta, 1933); and just as we have found that males are induced by crowding we find that excessive crowding (or otherwise induced excessive retardation of the production of parthenogenetic eggs) limits or inhibits male production. Then in nature maximum male production should occur when the Cladocera population becomes dense but decrease with excessive density of population, and in the latter population phase with the accompanying paucity of food, sexual eggs should be produced. So far as we have been able to make appropriate observations in nature this is precisely what we have found to occur.

Males begin to appear in a wild population when the population is dense, but before scarcity of food is apparent in a reduction in the productivity per mother. At first there are only a scattering few males, but almost immediately thereafter great numbers may appear.

Concomitant with or quite immediately following the very acute stage of male production in a wild population some females produce sexual eggs (haploid and requiring fertilization); with increasing food scarcity sexual-egg production soon becomes abundant, sometimes even exclusive.[1]

[1] With Daphnia sexual or parthenogenetic eggs may be produced in any clutch depending upon environmental conditions (Banta, 1925). With Moina sexual eggs may appear as the first clutch and a subsequent clutch is either

During the acute stage of sexuality in one race of Daphnia occasionally every female will be found bearing sexual eggs; or most individuals bearing sexual eggs and the others with only male-producing parthenogenetic eggs.[2] The peak in male production, however, briefly antedates the great prevalence of sexual-egg production. This sequence ensures the simultaneous occurrence of adult functional males and sexual eggs.

With Moina in nature, so far as we have observed them, the sexual reproductive phase is of only 4 to 9 days duration, after which the population of active Moina, at least usually, entirely disappears, even though the pond, although fairly low in water content, does not become dried up. With Daphnia the sexual phase of reproduction is usually less intense and less brief. It is followed by a great decrease in Daphnia population, but, unless the pond is very low or actually becomes eliminated by drying up, some individuals ordinarily survive and continue the race by parthenogenesis.

The adaptive value of this sequence is so obvious as scarcely to require mention. Surface water ponds, to which the situation I have been describing particularly applies, are well filled in early spring. With the season's rise in temperature, bacteria and other protophyta, upon which Cladocera mostly feed, rapidly increase in numbers—even more rapidly for a time than do the Cladocera themselves. Food conditions, temperature, pH, etc., in the pond are exceedingly favorable for rapid Cladocera multiplication. At a somewhat later time the great abundance of food organisms, together with further rise in temperature, still further accelerates the increase in

sexual or parthenogenetic (in accordance with immediately preceding environmental conditions). But with Moina sexual egg production never follows parthenogenetic eggs in an individual and any individual which is once parthenogenetic continues so. Only a first clutch from a given female and immediately succeeding clutches may be sexual eggs.

[2] I have never observed a situation with Moina in the wild in which all parthenogenetic young being produced were males. Possibly our laboratory average of about 40 per cent. male production with crowding is near the proportion which occurs in nature.

the already substantial Cladocera population. Soon there comes a time when the Cladocera population has reached the maximum which permits full fecundity. Fecundity then decreases, doubtless being influenced by the failure of the organisms, which serve as food for Cladocera, to sustain their former rate of increase, probably both because of their rapid consumption by Cladocera and because of depletion of the pond water constituents upon which they are in turn dependent. Judged by our laboratory experience fecundity is also influenced by another factor—the density of by-products of the Cladocera and other animals in the water of the pond.

Observations in nature indicate that it is shortly before fecundity begins to decrease that male production by the Cladocera population starts to occur and to occur almost simultaneously among all the types of Cladocera resident in the pond. The prodigious rate of increase continuing, density of population increases very rapidly. Male production is soon at its height and then presumably falls off (observations in nature have not been fully directed toward this point). Males become sexually mature in from 3 to 5 days, by which time acute scarcity of food has ordinarily supervened and sexual eggs appear—a few at first, then almost immediately vast numbers. This cycle of events, so far as observed, occurs while the pond is rapidly receding in volume of water, thus further accentuating population density.

Soon following the stage of acute density of population, scarcity of food, production of sexual eggs and presumably accompanying a rapid decline in pH, the pond water changes in its appearance from a slightly (or more than slightly) cloudy or hazy appearance to a clear appearance without visible indication of suspended particles. This changed appearance of the water is very striking. As this clearing of the water progresses, the Cladocera population undergoes a sudden decline—Daphnia become exceedingly scarce or disappear; Moina entirely disappear. Often such a pond becomes

dried up within a few days. If it does not become dried up, the cloudy appearance is gradually restored. If it becomes markedly replenished by the run-in of surface water from heavy rains, the slightly cloudy appearance so favorable for the types of Cladocera under discussion is soon restored. This characteristic favorable cloudiness is not that produced by silt but is caused by bacteria, etc., in suspension.

With the return of the favorable cloudy appearance of the water Daphnia become more abundant. Moina may reappear, due to the hatching of some of the sexual eggs. The increase of Daphnia population may also be due in part to hatching of some of the sexual eggs, but it is also due to the production of parthenogenetic young by the survivors, if any, of the crisis through which the Daphnia have recently passed.

Although we have not seen them, later, presumably second, periods of sexual reproduction have been observed in natural ponds in late summer or in autumn (Wesenberg-Lund, 1908; Berg, 1929; Berg, 1931). Male reproduction in later autumn is probably stimulated both by population density and by lowered temperature. With some Cladocera forms it seems not at all improbable that two or even more of these environmentally-controlled sexual periods may occasionally arise within a season—contingent upon the appropriate sequence of physical and biological conditions in the environment.

A curious phenomenon occasionally observed with Daphnia in nature is of interest. Daphnia are usually rather generally distributed within a pond, so that a single sample taken with a net is likely to reveal the characteristic forms present. I have on a few occasions, however, found Daphnia grouped together by the thousands in dense aggregations, 3 to perhaps 20 feet in diameter, and the population of this particular type elsewhere in the same pond very sparse. Those in the aggregations maintain their positions within the group by their

characteristic swimming activities. Males, and I think also sexual eggs, occur within such an aggregation (though I do not definitely remember concerning the sexual eggs). It would seem that occasionally environmental conditions arise which stimulate these animals to come together in close groups and that within these aggregations conditions appropriate for male production and for sexual egg production arise.

It would appear from such observations that in a pond in which there was insufficient suitable organic matter to develop a great abundance of food organisms upon which Daphnia thrive, a general density of Cladocera population sufficient to stimulate male production might not arise. Under this contingency aggregations of the Daphnia population, induced by unrecognized environmental conditions, might provide the stimulus for male production which otherwise would fail to occur. These last two statements, while based largely upon surmise, appear to fit in both with facts learned from laboratory experiments and with observations in nature.

It thus appears that with Cladocera, population density plays an important rôle in the fundamental life cycle of the race—a rôle shared by low temperature and probably by other environmental conditions—but a rôle largely taken by conditions associated with a dense population. The fertilized winter egg in many, perhaps in the majority of, natural situations in which Cladocera occur is essential to carrying over the race through winter or drought. Population density directly gives rise to the fundamentally adventitious males, and scarcity of food, ordinarily resulting from over-population, likewise causes the production of the sexual eggs. Population density even *times* the coincident occurrence of sexually mature males and eggs ready for fertilization. Under conditions where general population density does not arise, occasionally these animals spontaneously aggregate with presumably a like result—the occurrence of males, sexual eggs and the consequent fertilized winter egg adapted for

carrying the race over ordinary natural contingencies, such as desiccation or freezing.

I made reference earlier in this paper to the fact that with many animals equal numbers of the two sexes seem biologically unnecessary. We have seen that in Cladocera males occur only under conditions of at least moderate stress—dense population, low temperature, etc.—but that in most cases, inasmuch as fertilized sexual eggs are necessary to carry the race over winter or the drying up of a habitat, these males are essential to the continuation of the race.

But this is only part of the story. Some races of organisms have still further subordinated the reproductively inferior male. Indeed certain species or races have adequately solved the problem of the, for them, useless male by completely eliminating the male sex.

A race of Daphnia has been found in the Arctics in which no males were seen (Olafsson, 1918). This presumably pan-parthenogenetic race not only has parthenogenetic summer eggs but produces winter eggs also apparently parthenogenetic, and these latter are capable of developing after withstanding the rigors of the long Arctic winter. A similar race has been found at Cold Spring Harbor (Banta, 1925). We have been unable to find males of the Cold Spring Harbor race in nature or to cause their production in the laboratory; males certainly do not function and probably do not occur. We have demonstrated that in this race the so-called winter eggs (or Dauerei of the Germans) actually hatch without fertilization.[3]

Certain lacustrine races of Daphnia apparently are quite without males.[4] These lacustrine maleless races, so

[3] Dr. Franz Schrader (1925) has determined that these winter eggs, like the summer parthenogenetic eggs of Cladocera, *have only one maturation division and remain diploid.*

[4] It is most interesting to note that Dr. Kaj Berg (1931) has been able to induce male production in one such race, indicating that in this race, unlike the Cold Spring Harbor race, the capacity for male production has not entirely disappeared.

far as I know, are confined to larger permanent bodies of water in mild climates. In another group of animals, Kinsey (1920), Patterson (1928) and others have pointed out that some gall-inhabiting wasps, popularly called "gall flies," reproduce solely by parthenogenesis; certain other species of these same gall-producing insects sometimes produce a few functionless males which appear to be without the sexual instinct. Other illustrations of maleless races of animals could be cited.

Now sexual reproduction, with its accompanying ready redistribution of the different hereditary factors, has been considered and probably is an important factor in producing and refining the adaptations of animals. The variations thus produced may logically seem to facilitate adjustment to a changing environment or make possible successful migration into new environments. But the swamping effects of cross-breeding on the other hand largely discount the advantages of sexual reproduction as a method of perpetuation of advantageous combinations of characters.

Our studies have shown that mutations occur in parthenogenesis and that these mutations are possibly as diverse in their nature as the more generally studied mutations in sexually reproducing forms (Banta and Wood, 1928; 1932). In parthenogenetic reproduction there is none of the kaleidoscopic recombination of characters among offspring such as occurs in sexual reproduction. All the descendants of an individual possessing a certain combination of characters have the identical character complex of the original progenitor, except as a further mutation occurs. Hence an advantageous combination of characters or a favorable mutation once it occurred, barring accident, should continue and come to prevail in the habitat in which it is advantageous. The chances of its becoming lost or submerged are immensely less than with sexual reproduction.

Hence it is easy to conceive (1) that in the economy of nature a largely parthenogenetic or a wholly partheno-

genetic, thelytokous race may have a biological (reproductive) advantage under conditions of severe competition; (2) that from the genetic standpoint it may at most suffer no handicap; and (3) that from the evolutionary point of view it may actually be more advanced than its bi-sexual sister races. Known maleless or nearly maleless races are found to occur and presumably have become evolved under conditions of crowding or dense population. One can readily arrange a series of present-day Cladocera races in order of decreasing dependence upon sexual reproduction and upon the male sex (Banta, 1930). In what may logically be considered the most advanced races in this series males do not occur, and even the functional winter egg is produced without male intervention.

Considering the obvious advantages of parthenogenesis —the reproductive superiority of an all-female race which is quickly able to produce a maximum population and thus fully utilize (not designedly of course!) an advantageous situation—together with the apparent evolutionary tendency toward elimination of the male sex among animals inhabiting a restricted or temporarily favorable habitat, one may surmise that further races of animals may readily evolve in the direction of an extension of parthenogenesis and of male subordination or elimination. Such has occurred with animals which live in habitats restricted either in locale (as Miastor in a decayed log) or time (as Daphnia in a temporary pond) and under conditions in which density of population appears to control male production. Inasmuch as thelytokous forms have arisen in such situations, it seems probable that the evolution of such types is influenced by population density and that further evolution of such races may readily occur.

Thus it appears that density of population not only controls sex ratios in Cladocera, but that it also promotes the evolution of thelytokous races; and that possibly the ultimate in evolution as concerns reproductive processes in certain lower animals is the *zero* sex ratio—a maleless race.

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ON BIOLOGICAL PRINCIPLES AFFECTING POPULATIONS: HUMAN AND OTHER[1]

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I

ONE of the most striking and novel aspects of the marked recrudescence of interest in the problems of population that began about a quarter of a century ago was the emphasis put upon the experimental attack on such problems. Since it was naively thought then that human populations were not amenable to experimental treatment, a distinction was drawn between populations of men and those of lower animals that could be experimentally handled. But all this has now been changed, as have so many other aspects of life to which we had become so comfortably habituated. We have been shown with devastating clarity that a great human population may be subjected to experimentation just as easily and ruthlessly as a *Drosophila* population in a pint bottle, and far more irresponsibly than would be tolerated in any respectable biological laboratory.

But it is doubtful that the distinction was ever really as fundamental biologically as it has sometimes been thought to be, for nature, as its normal way, has set up the equivalents of long-term experiments by permitting human populations to exist under widely divergent circumstances, the consequences of which may be recorded and analyzed by appropriate statistical procedures. Thus in India we may observe what happens in a human population, wherein approximately one half the individuals born die before reaching the age of puberty and the consequent power to reproduce; where the food supply produced is sufficient for only two thirds of the population trying to live on it; where contraception is but little prac-

[1] From the Department of Biology of the School of Hygiene and Public Health, The Johns Hopkins University.

tised; but where the rate of increase of the population is nevertheless such that in 1930 the ratio of workers to non-working dependents was as 44 to 56. To compare with such a situation as this Denmark might be considered, where one half of all the individuals born live to ages well on into the seventh decade of life, long past the period of reproductive capacity in the females and practically so in the males so far as concerns statistically significant reproductive results; where the food supply produced is so far in excess of the needs of the population that a substantial part of the national wealth is derived from its exportation; where contraception is well understood and extensively practiced; and where the average annual growth rate per cent. is 0.68, which is only about a half that of India. These examples sufficiently illustrate the fact of the enormous range of diversity in the general settings in which human populations exist, on the one hand, and the great extent and particularity of the statistical information about them routinely collected and recorded nowadays in all reasonably advanced countries, on the other hand. It is in fact possible to analyze many of the biological phenomena exhibited by human populations in a manner comparable in precision to what is done with lower forms through laboratory experimentation.

During the last fifteen years a great deal of intensive work of really penetrating character has been done on the problems of population from both sides, human and infrahuman. Out of this work has emerged a broad generalization that is becoming more solidly grounded with each passing year's crop of new results. It is that the biological principles underlying population phenomena are fundamentally identical in man and in other animals. Such differences as there are concern, with one exception, relatively unimportant details—anatomical, physiological, psychological and environmental. Beneath these differences are the basic biological phenomena common to all, upon which a real understanding of population processes must in the end rest. It surely is no accident that the normal growth of populations of men and of a wide range

of extremely diverse lower organisms is accurately described by the same general type of curve, the logistic. When particular populations diverge in their growth from this type of curve, experience has shown that it is usually possible to account reasonably for this divergence by the operation of circumstances and forces peculiar to the particular case, either in time or in space or both.

The one significant difference in the basic biology of human and infra-human population is behavioristic, with slowly increasing quantitative consequences. Amongst organisms lower than man it is in general true that sexual activity is regularly and inevitably followed by reproduction, during that part of the total life span in which reproduction is physiologically possible. Man, on the other hand, long ago learned that the normal functioning of his reproductive mechanisms could be tampered with in a variety of ways, having as their common end the reduction of the probability of conception and reproduction without serious diminution of the pleasures of sexual congress. Just how important this indubitably real difference between man and his animal forebears is for his present and future population biology it is impossible now to say with any great generality or precision. But it is the subject of the most active investigative efforts of the students of human population problems at the present time, and real progress is being made in its scientific appraisal.

I'

A brief analysis, but as general as possible, of the basic biology of population phenomena should help to a better understanding of two things: first, the mutual relationship, convergent and divergent, of the two lines of investigative approach, human (statistical) and infra-human (experimental); and, second, the sort of problems that in future work may more profitably be attacked from one or the other side.

A population is a group of living individuals set in a frame that is limited and defined in respect of both time and space. The biology of populations is consequently a

division or department of group biology in general. The essential and differentiating feature of group biology is that it considers groups as wholes. It aims to describe the attributes and behavior of a group as such, that is as an entity in itself, and not as the simple sum of the separate attributes and behavior of the single individual organisms that together make up the group. The concept of group attributes, separate and different from the attributes of the component individuals, is a familiar one in other fields. For example, the familiar measures of variation such as the standard deviation and coefficient of variation, are quantitative expressions of a group attribute, namely, the shape of the distribution of frequency of the component individuals in respect of the character measured. Similarly, birth rates and death rates are quantitative expressions of group attributes, meaningless relative to any individual.

In general, it is plain without further elaboration that the concept of self-contained and self-sufficient group attributes is a statistical concept—an abstraction that in truth is one of the foundation stones of the whole superstructure of statistical reasoning and methodology. A relevant consequence for our present theme is that whether one studies populations of lower organisms experimentally, or studies human populations, or indeed studies any kind of populations by any sort of technique, it will be impossible to avoid the use of statistical methods and modes of reasoning. In this respect all students of population, whether sociologists, economists or biologists, are on the same footing and can not escape the consequences. Broadly speaking, it seems probably true that, on the whole, the students of human population have had a deeper and wider knowledge and understanding of statistical methodology than the laboratory experimenters in the field of population have had, including the intangible kind of knowledge often not capable of very precise expression but none the less real and important that comes only from long experience with a material and is in some part reached by a process sometimes facetiously referred

to as osmosis. If this is so, and it seems to be, the experimenters are likely to be done no serious or irreparable damage if they rather thoroughly familiarize themselves with the work of some of the major prophets in the field of research on human populations—such men as Farr, Yule, Quetelet, Körösi, Kiaer, Bertillon, Willcox, Sydenstricker, Dublin and Lotka, to name but a few representatives chosen in part because of the range of diversity of their particular interests.

The purely biological attributes or characteristics of populations as whole groups, whether of men or mice, that are directly relevant to the discussion and elucidation of significant population problems are not numerous. In the first line of importance there are but three of them. These are size, growth and quality. Each may be briefly considered separately.

(1) *Size* of populations may be considered in either absolute or relative terms. It is the total absolute number of individuals composing a given population at a particular time, or the number of individuals at that time in proportion to the size of the spatial framework in which the population exists. The most significant method of measuring relative population size or population density will of course differ according to the habits and ecology of the species concerned.

(2) *Growth* of population is measured by the change of size with time in either the positive or negative direction. The increase or decline of populations in size with the passage of time is, to the special student of population problems, easily the most interesting biological phenomenon they exhibit. It rests upon the fundamental variables of fecundity, fertility and life duration, and takes the investigator at once into the fascinating realm of the physiology of reproduction, that has been until recently the most neglected part of all physiology. In human populations the rate of growth in a particular period is closely correlated with social and economic behavior and in extremely complex ways. History plainly shows that the rate of population growth—positive or negative—has

been a factor of no mean importance in shaping national policies and influencing political conduct. In infra-human populations in Nature the rate of population growth at a particular time may be associated with abrupt changes in habits and behavior of great significance evolutionally, particularly in relation to what is called the "Balance of Nature." This expression "Balance of Nature" is, of course, only a figurative way of speaking in general terms about the relative sizes of the populations of different species existing in the same spatial framework at the same time.

(3) *Quality* is a convenient term to designate the genetic constitution of the population, or, put in another way, the frequency distribution in absolute or relative terms of the qualitatively different genes represented in the group. In human populations this attribute has come during the last half century to be regarded as of prime importance. It is what the eugenists fight and bleed about, as well as preach and pray over. It is like the weather in at least the respect that people discuss it a lot but seldom really do much about it. The reason why is at bottom about the same in the two cases. The problem presented is somewhat too big a bite for our feeble masticatory powers. Here the laboratory experimenter with animal populations has one of his greatest advantages. He can make the genetic constitution of his populations precisely what he wants it to be and hold it there. In the long run much useful knowledge to the whole enterprise of population study will surely accrue from this advantage.

It is evident that these three biological attributes of populations are not separate and disparate things. On the contrary, they are in fact integrally interconnected with each other. The size of a population at any given time depends upon its past growth—is in fact the static aspect of that dynamic phenomenon. Both are reflections of the biological quality of the population—its genetic constitution. But this interconnection and integration is nothing peculiar to population biology. It is one of the most characteristic features of all biological events and living organisms.

There are other group attributes of populations that might be listed besides these three. But none seems to be of the same order of significance, so far as may be judged in the present state of knowledge. Group behavior or psychology is the one omitted that most insistently demands to be discussed. A great deal is heard nowadays about "mass," "social" and "racial" psychology. But what emerges is not very precise, or capable of quantitative measurement, analysis or appraisal. Rather it suggests to the biologist a rather loose and vague *congeries* of ideas mainly reached by somewhat dubious analogical sorts of reasoning; making entertaining reading, but seeming to resemble perhaps a little more closely the science of the movie theater than that of the Cavendish Laboratory. A single example may serve to illustrate the point. The behavior of lynching parties is a, if not *the,* classical example of so-called "mob psychology." Soft-headed sentimentalists say that under emotional stress the mob acts as a unified whole and that therefore no single individual in it should be held responsible for what happens. An old friend of mine during his early life as a reporter had the somewhat ghastly experience of witnessing in his professional capacity a considerable number of lynchings. From his observations he made three weighty generalizations, to wit: (1) that the activity of every lynching mob could have been instantly stopped at any stage of the proceedings by the forcible apprehension there and then of one particular single individual member of the mob; (2) that it could have been stopped at any stage by one single individual not a member of the mob who had the intelligence, courage, personality and desire to do so; (3) that a large, though not precisely determinable proportion of the mob in every case was heavily alcoholized at the time. Like all successful reporters my informant was a trained and reasonably accurate observer. Every intelligent eye-witness of a lynching I have been able to question agrees unreservedly with his generalizations, and realistic reports of such orgies confirm the soundness of the conclusions. If these generalizations

be accepted as at least an approximation to reality, what becomes of the concept of "mob psychology" so far as concerns lynching as a group attribute in any precise scientific sense—the sense in which rate of growth is a biological attribute of a population?

In sum, if there is any desire or intention to keep the investigation of population problems on a rigorously scientific footing it seems wisest to leave aside for the present the discussion of psychological group attributes until some future time when they may perhaps become more precisely understood.

III

The most commonly observed, and therefore reasonably to be regarded as the normal, tendency of infra-human populations of all sorts, the physical environment being relatively constant, is to increase in size by growth to an upper limiting value, and thereafter slowly oscillate up and down over a small range about that size. The absolute magnitude of this upper limiting value is plainly in general terms a function of at least two variables, the species concerned and the size of the spatial framework in which the population exists. But beyond this general fact extremely little is as yet precisely known. What is it that makes populations slow up in their growth rate and asymptotically approach a limiting size? This is surely one of the central unsolved problems of population biology. And it is an extremely complex and difficult one. The idea that it is a consequence of a simple relation between means of subsistence and size of population, as Malthus was inclined to think, will do no better for population growth than it will for the growth of an individual. Human beings stop growing in size in the early twenties. But plainly this is not due to lack of food nor, conversely, can any known plan of abundant feeding produce men ten feet tall. One of the more elegant generalizations of biology states that man stops growing because he is a form characterized by limited growth, which once more demonstrates the charm of sonority as a surrogate for pro-

fundity. On the population side it is a matter of plain fact that the population of the United States is slowing markedly in its rate of growth as it approaches the asymptote. But surely no one would allege that simple pressure on means of subsistence is a factor of any importance in causing this slowing growth, particularly in a country that produces a surplus of food for export even while it fatuously pays farmers for not growing hogs, wheat and various other useful things, and in which the evidence produced during the depression years shows that those parts of the population less abundantly nourished have exhibited increased rather than diminished net fertility rates. This latter finding, by the way, would have greatly cheered those earlier worthies in the field of population research, Thomas Doubleday and Herbert Spencer.

Human populations, however, really offer little help in analyzing this problem. The difficulty is that there have been no observations over a long period of time on a human population near its asymptotic limit. All human populations at the present time of which there are records are either growing or declining, or have very recently closely approached what appears to be a limiting value. Demographers and students of the mathematical theory of population have contributed valuable discussions of "stable" populations, in which deaths just balance births and the size of the population stays constant. But so far this remains a nearly pure abstraction, unsupported by any large or long-continued body of actually observed data. Why this should be the fact is not quite clear. A great many human populations for which data are available started the cycle of logistic growth in which they now are, from a lower asymptotic value a long way above zero. Had these populations for a long time prior to the beginning of their present cycles stood steadily at, or slightly oscillated about, the lower asymptotic levels from which these cycles start, or had they been at those levels only a short time before the new cycles got under way? There are no reliable data from which these questions can be answered with any generality. The numerical history of human populations is extremely short.

But if the time span of man's population history is brief, his population pre-history is long. And from it emerges a baffling puzzle. The present logistic cycle of world population growth got discernibly under way at about the middle of the seventeenth century—say 300 years ago in round figures—and started from a lower asymptotic value of about 445 millions (*Human Biology*, 8; 399–419, 1936). This figure is, of course, only an estimate, but can not be far away from what was the actual fact. Since then it has increased to about 2,073 millions, again in round figures, as of an average date of 1931.5. In other words, there has been in roughly 300 years a 4.7-fold multiplication of human beings on the face of the earth, and this present cycle of growth appears to be only about two thirds completed. But man as a distinct and differentiated species had been on the earth at least 100,000 years before 1630, by the most conservative anthropological estimate yet suggested for serious consideration, and probably nearer 500,000 years. For the sake of the argument, however, and because it is so conservative as to be quite inoffensive to anybody, let us take 100,000 years as the time period. Yet the world population in 1630 was only a paltry 445 million, a density of a little over 8 persons per square mile as against the present 40. This being so we must necessarily conclude either (a) that the human populations of this globe grew at an extremely slow rate for 100,000 years as compared with the performance of any populations now existing; or (b) that for a long time—thousands of years— prior to the seventeenth century the population of the world stood stable at between roughly four and five hundred million, or oscillated in waves of small amplitude about some such figure, this representing the relatively stable upper asymptotic level of a cycle of population growth consummated long before; or, finally, (c) that during some period or periods in the vast span of 100,000 or more years of man's life on the earth the world population was much higher than 445 million, and subsequently lessened, for reasons wholly unknown, to reach that figure when reasonably reliable population history begins.

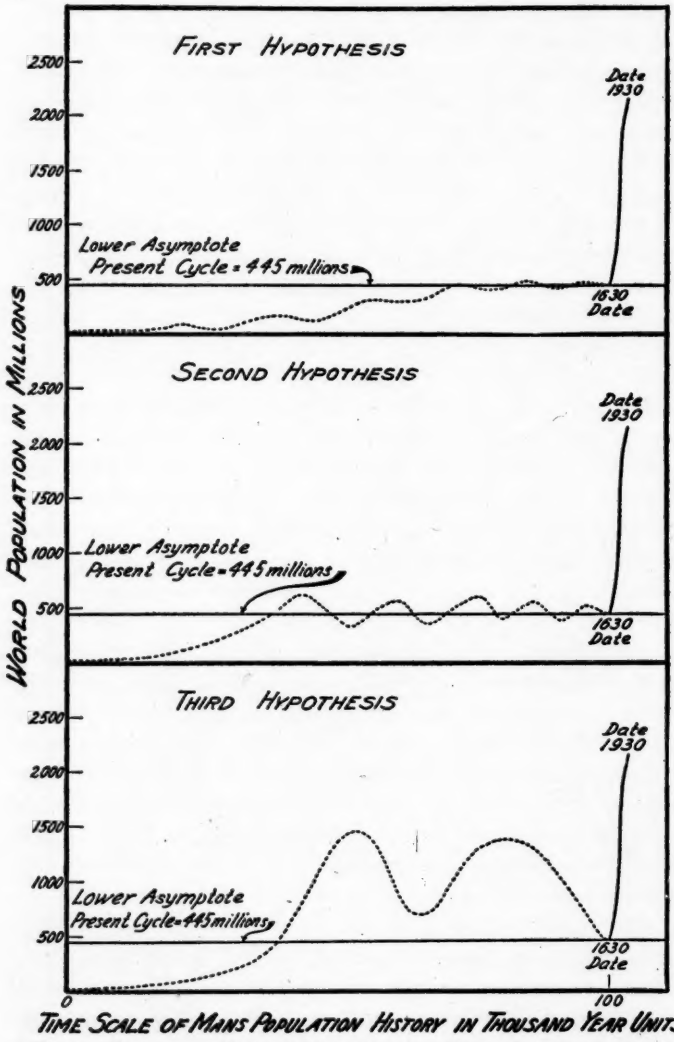

FIG. 1. Diagram to illustrate alternative hypotheses as to the history of the human population of the earth. The portions of the curve drawn in broken lines are purely hypothetical, except as to the total time scale. The portions of the curves depicted by a continuous solid line (1630–1930) represent the known facts as to world population growth, and are plotted to the same time scale as the earlier period.

In order to clarify these three alternative hypotheses for the reader, Fig. 1 is inserted. It exhibits them in graphic form, drawn to scale.

Which of these three alternatives is true? No one can say with any authority. But all the relevant evidence suggests that there were more human beings living on the face of the earth in the middle of the seventeenth century than there ever had been at any prior time since man set up in business for himself. Furthermore, it appears probable that if the population of the world had been stabilized for even 2,000 years before the seventeenth century we should have heard about it in historical writings. Instead the references and inferences about population that can be drawn from ancient history indicate it as growing, often slowly and irregularly to be sure, but still growing.

So then we are left with alternative (a), a slow time rate of growth of world population over a long time prior to the Middle Ages, let us say, followed by a relatively terrific spurt of growth not yet ended. How is this to be explained? The first suggestion that comes to mind is that the primary cause behind the new cycle of logistic growth of world population that started in the seventeenth century was that mankind became aware about that time that its effective universe was expanding. The earth was not growing physically bigger, to be sure; there were no new acres of land being really added to the planet. But many were being discovered, uncovered and put to use, and in general man's *effective* universe for purposes of living certainly was expanding; and at an accelerating rate. This was being brought about by new discoveries and new ideas that made it possible for man to exploit far more effectively than he had known how to before the natural resources for human living inherent in the earth on which he lived. It is not possible to develop this point in detail here. But one of the most important and obvious aspects of the expansive changes in man's effective universe that have appeared in the last 300 years is the ever-

increasing facility of communication and transportation, with its consequent broadening of the base and acceleration of the tempo of trade. Indeed it was the development of railroads that had a large part in making the gloomy prophecies of Malthus about the future of mankind at the end of the eighteenth century seem comically absurd soon after he made them. Another change of similar sort was the development of relatively cheap power, associated primarily with the discovery and rapid improvement of practicable steam engines. A third was the development of modern scientific agriculture, with its enormously increased average yields. Finally, a fourth element was the development of knowledge of the principles of public health and sanitation.

In sum, what expanded man's effective universe and made possible a great spurt of population growth was scientific discovery and the application of its results (in the broadest sense and including exploration as well as technology) to man's basic problem of how best to cope with and exploit his natural environment.

There is much evidence to support this view as an explanation of what is known of the whole history of human population on the earth. It would take a sizable book to review it. Only one striking feature of this mass of evidence can be mentioned briefly here. It is that, to this day, peoples who, because of geographical location, climatic conditions, general stupidity or other causes, have been unable to participate in any real sense in the consequences of an expanding effective universe, are still growing in population very slowly if at all.

But even if this suggested interpretation of the history of world population be accepted as in some sort a rational explanation of the facts, it obviously gives little real insight into the basic biology of the processes involved. As has been intimated before, it is the experimenters with infra-human populations who must do the fundamental work here. Theirs is the task to find out the biological causes that alter birth and death rates in a growing population and lead it finally to a stable, or relatively stable,

limiting size. Something has already been learned about this problem, but much remains to be done. Particularly the effect of density of population *per se* upon natality and mortality has been intensively studied in various forms by different workers. In one species, *Drosophila,* the analysis of the effects of this variable has been carried to a point where it has been possible to appraise with considerable precision the influence upon ovulation and fertility of one factor in population density, namely, the so-called "collision" or interference effect. This matter has, however, been discussed in detail by Dr. Park in his contribution to this symposium and therefore need not be further elaborated here.

Before leaving the discussion of the history of human population one further point may be mentioned. Zoologists have long been familiar with the phenomenon of periodic rapid multiplication of populations of lower mammals—lemmings being the classic example—to such enormous absolute sizes as to entail high densities of population within quite large, but still limited habitat areas. These great spurts of lush reproductivity end, after the density has reached a limiting height, in self-engendered destruction of the major part of the population, usually through rapid and undirected mass migratory movements out of the overpopulated area. These mass migrations blindly push on until some obstacle like the sea or a river is reached, in which vast hordes of the mass migrants perish. The essential biological elements concerned in this series of events appear to be; first, in a restricted habitat area, absolutely large or small as the case may be, there exists a population that has maintained itself at a relatively constant or very slowly growing level for a long time, as a consequence of the operation of natural checks physical or biological or both; second, during a relatively short period on the total time scale of the species—say a few seasons—these natural checks are lifted or greatly abated and living becomes much easier, which means a relatively sudden and large expansion of the effective universe for the species under discussion;

third, this expansion of the effective universe is promptly followed by an almost explosive spurt of population growth; fourth, the increasing density of population that follows presently reaches a point where there impinges upon the component individuals a set of stimuli that lead to disturbance reactions, so generally participated in as to give the semblance of a mass reaction of the whole population. Fifth, these reactions, reinforced by the habits of cohesive gregariousness that appear to be a wide-spread characteristic of mammals generally under conditions of environmental and emotional stress, lead to undirected mass migratory movements and ultimate destruction of major parts of the population.

With this picture before us may I venture to suggest—as pure speculation at the moment, but nevertheless possibly worthy of some consideration—that the course of events relative to human population since man first emerged as a distinct species presents objectively a rather remarkable parallelism to the successive stages (up to the final one) in the history of a lemming population that has been depicted. For thousands upon thousands of years the human population of the earth grew slowly, because the conditions necessary to more rapid growth did not exist. Then about 300 years ago, the advancement of learning suddenly expanded man's effective universe and has kept on expanding it. There has followed a spurt of population growth of an explosiveness that is seen, when plotted to a proper time scale, to be comparable to that of an epidemic. This has produced a density of 40 persons per square mile for every single square mile of the land area of the earth—good, bad and indifferent land all counted in. That there are associated with this present density stimuli producing sensations of discomfort seems scarcely open to argument. Can it be honestly denied that on a world-wide view, *unrest* is the dominant characteristic of human behavior to-day? And behavioristically viewed, unrest is surely the cardinal symptom of discomfort.

Up to this point the parallelism between the two cases seems reasonably evident. Not being a prophet I have no desire to push it further. But I do venture to suggest that it merits thought. Different species react in different ways to similar stimuli, especially in the emotional field. One scarcely envisages mankind marching to a watery grave just behind a horde of frantic lemmings. But does any one find it difficult to conceive of men marching off in the not too distant future to a war? Or to doubt that, once well started, that war will entangle in its meshes the major portion of mankind before it is finished? Or, finally, to doubt that the next world war will achieve a destructiveness hitherto undreamed of in the wildest flight of imagination? The experience of 1914 to 1918 taught the military men of all nations one thing they had not grasped before—how to make war really destructive. And every war office in every land has been developing that theme contrapuntally ever since.

The thought is a gloomy and horrid one. Perhaps it is essentially false. Perhaps mankind will in the next few months evolve techniques of living happily and contentedly at high population densities in a physically limited universe. But plainly he hasn't yet and the failure to has not been for lack of trying. The problem of population is a difficult and serious one—perhaps in all its implications the most serious one facing humanity to-day. There is real need for a broader and deeper understanding of its underlying principles. Slowly but surely the studies of the ecologists on natural populations and of the laboratory workers with experimental populations are contributing to such an understanding.

IV

Experimental studies of infra-human populations have been the target for a good deal of light-hearted and sometimes contemptuous criticism from some students of human population problems, and especially from some sociologists and economists. This was to be expected.

In all fairness could biologists possibly hope or expect that ignorance, narrow-mindedness, bigotry, dogmatism and jealousy should be *their* exclusive and permanent prerogative? After all, the fact is that the biologists muscled in on the population racket just a few years ago, to put the matter in appropriate modernistic terms. For a long time it had been cultivated as the exclusive bailiwick of another gang. Is it remarkable that they are peeved at predatory poachers?

Actually, though the matter has been introduced in an oblique manner with inverse emphasis, it is a patent fact for all to see that biologists are as a group singularly, indeed almost miraculously, free from the usual intellectual and moral disabilities and vices so common to the rest of mankind. Indeed it is one of the most extraordinary phenomena of organic evolution, when soberly considered, that it should have produced a homogeneous group as purely and unselfishly devoted to the search for truth as the biologists are. *We* are open-minded and always receive new truths gladly, whether they fit into the pattern of congealed and desiccated wisdom that we cherish, or do not. We, of course, know and admit this; but could a sadder example of general pyxillation be found anywhere than is revealed in the fact that the sociologists and economists feel precisely the same way about themselves?

The efforts of the biological experimentalists in the field of population study are most commonly met from the human side with the supposedly devastating point that since man is a much more complex creature than a fly or a beetle or a yeast cell it follows that their results can not possibly have any significance for the elucidation of the problems of human populations. This contention reminds one of the suggestion of a late Pope to Lord Odo Russell that it was a theological error to suppose that "Christians owed any duties" to lower forms of life. In point of fact, the fundamental problems of population are biological problems, and always have been, because they are a part of the much broader and deeper problem of organic evolu-

tion. It has been said, and pregnantly, that in final analysis the problem of evolution is a problem of birth rates and death rates, *et praeterea nihil*. That biologists neglected the field of population so long as they did is the fault of no one but themselves. It always really belonged to them, by right of its true nature and content. Some parts of it, in the nature of the case, can never be successfully or penetratingly approached with human material. These acres can be tilled only by the experimentalists.

It is further said that economic variables are among the most important in human population phenomena, that animal populations are not affected by such variables, and that therefore it is silly to suppose that the behavior of such populations can have any meaning for the sociologist. The major premise of this contention is obviously true. The minor premise and the conclusion are not. For what are these sacrosanct "economic variables" when viewed from the philosophic position of theoretical biology? Plainly nothing other or more than certain significant elements in the effective environment of one species, *Homo sapiens*. Their *equivalents* are found in the effective environments of other species, and indeed the influences of these equivalents in relation to population problems are now being actively studied by Lotka, Gause, Volterra and others. They are being studied, furthermore, by the precise methods of the natural sciences; not by the more hedonistic technique of analogical verbiage, with its love for "values," "trends," "standards of living" and other such ill-defined concepts.

Mutual understanding is one of the major difficulties under which all animals labor, and the difficulty seems to be greatest in man, even though—or perhaps because—he has the biggest brain. Therefore it is important to make it clear at this point that no biologist supposes or suggests that immigration laws or the removal of legal barriers to the free dissemination of contraceptive information or governmental population policies generally are going to be consciously formulated upon the basis of the results achieved by students of experimental fly, beetle or other

infra-human populations. Indeed it is probable, so keen and ready is the intelligence of these students, that if one of them had a suspicion that his work was to be perverted by some politician to such uses, he would promptly quit population study and turn to some such innocuous and reliable old standby of a problem as the metamerism of the vertebrate skull. Yet, parenthetically, it may be noted in passing that no insignificant part of the governmental policies imposed upon their bewildered populations by Nazi Germany, Soviet Russia and Fascist Italy, are direct, conscious and overt applications to mankind of what are held to be sound inferences from experimental work with lower organisms in the field of Mendelian genetics. In fairness it should be said that this has come about for the most part without the connivance, or the support of the reasoned judgment, of the great leaders in experimental genetics. The phenomenon itself shows, however, that politicians are just as ready and eager to turn the results of experimental laboratory research as anything else to their own ends, in the pursuit of their "endless adventure," the gaining and holding of power over their fellow men in the name of government.

To return from this digression, the true situation at the present time is that no student of infra-human populations by experimental or ecological methods known to me has the slightest intention or desire to press his results forward into human affairs, or to make them bear any heavier burden than their just contribution towards the slow progression of knowledge about the principles of population generally. What all serious students of population problems are trying to do is to find out more about the general laws and principles that underlie and control population phenomena. The sociologist is not more sincere in this intention than the experimental biologist or the ecologist, nor less so. Neither group alone can solve all the problems involved. Each can contribute to the enterprise; each is essential to its successful prosecution. And in the long run each will find it helpful and nourishing to know a good deal about what the other is doing.

NEW EXPERIENCES WITH *OENOTHERA* MUT. *POLLICATA*[1]

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THE most characteristic feature of *Oenothera* mut. *pollicata* is the transformation of the hypanthium of the flower from a hollow tube traversed by the style to a solid pedicel intervening between the distal end of the ovary and the proximal end of the style. The first two individuals of this new type were seen in pedigree-family 31303, grown in 1932, as already reported (Shull, 1934). Two cultures in 1933 were derived from one of these original *pollicata* mutants, but one of these cultures (32278) contained three plants only, none of which was *pollicata,* and the other (32277) came to flower so late in the season that only a very limited amount of work could be undertaken to learn the location of the new gene, and its interactions with other genes in the course of development.

During the summer of 1934, I repeated culture 32277 under number 33115 and had five other cultures (33116 to 33120, respectively), derived at least in part from known *pollicata* parentage. These five new cultures consisted of one progeny (33116) from a selfed *pollicata;* one outcross (33117) of *pollicata* old-gold *pervirens* to *Lamarckiana* yellow "outside-in," two backcrosses (33118, 33120) of plants heterozygous for *pollicata,* to the homozygous recessive *pollicata;* and one selfing (33119) of a plant known to be heterozygous for *pollicata.* These cultures were undertaken for the specific purpose of locating the *pollicata* gene in relation to the other known mutant genes and of studying any peculiarities produced by the interaction of the *pollicata* gene with the genes of other traits; but since they have not yet reached their definitive goals, they will

[1] Read before the Genetics Society of America at Pittsburgh, Pa., December 29, 1934. A part of the cost of these experiments has been met from grants of the National Research Council, and the Bache Fund of the National Academy of Sciences.

not be discussed at this time, except to the extent of stating that as far as these cultures have gone they indicate that mut. *pollicata* is a typical Mendelian recessive, which is at least relatively independent of the *supplena* gene of the third linkage group with which it was associated at the time of its first discovery.

While waiting for these special cultures to run their appointed courses, I have been enabled by an unanticipated series of events to get an important part of the desired information much more promptly than could have been expected, and these new experiences provide the subject of the present communication. During the season of 1934 in no fewer than eight different cultures, not directly derived from a parentage known to include the *pollicata* gene, I have found new examples of mut. *pollicata* which have been produced in these families presumably as the result of crossing over. It is mainly through the situations revealed in these new manifestations of mut. *pollicata* that it is possible to report at so early a date that the gene of mut. *pollicata* is in the first linkage group where it is associated with the characteristic first linkage-group lethals, l_1, and l_2, by whose action it has been kept hidden undoubtedly through a number of generations.

The eight cultures in which these new crossover mutations have occurred will be considered, one by one, since they were being grown for diverse purposes, and any similarities and differences of the several families with one another were of an incidental nature.

(a) The first family in which a new appearance of mut. *pollicata* was observed was 33155, the progeny of a *Lamarckiana pervirens,* with old-gold double flowers, which had been given pollen of a *Lamarckiana rubricalyx* with single yellow flowers, but heterozygous for the mentioned three recessive traits, *pervirens,* old-gold and double. In brief, this represented a trihybrid backcross involving the three named recessive genes. Among the 25 plants of this progeny all but two of the eight possible permutations of the three genes were present, the missing

types being two of the crossover classes, double yellow *rubricalyx* and single yellow *pervirens*. In addition to the six expected classes, one plant was observed on July 31, 1934, to be a fine example of *pollicata* with *rubricalyx* buds and single yellow flowers.

(b) In family 33190, I found on August 3 two specimens of mut. *pollicata* among the 102 progeny of a selfed *Lamarckiana rubricalyx* with single yellow flowers, but heterozygous for the three recessive genes, *pervirens,* old-gold and double. On October 3, a third *pollicata* plant was found in this family, having the habit of *decipiens*. One plant of this progeny remained a rosette, and the rest had *rubricalyx* buds, but split in regard to flower color and flower form into 49 single yellow, 27 double yellow and 26 single old-gold, in essential agreement with the expected 2:1:1 ratio. Of the three unexpected *pollicata* plants, two were single yellow, the third a single old-gold. This distribution of the *pollicata* into both of the color classes, as well as the fact that the *pollicata* specimen in 33155 had yellow flowers, while the original *pollicata* had old-gold flowers, was in accord with the conclusion derived from the low incidence of the *pollicata* specimens in the original family in which it appeared, that the gene for *pollicata* is not in the third linkage group,—it is *not linked* with old-gold, though it was associated with old-gold in the original family in which it occurred.

(c) On September 18, I found a specimen of single yellow *pollicata* in family 33169, which was the progeny of a selfed *Lamarckiana pervirens* heterozygous for double. The 51 plants in this family were all yellow-flowered *Lamarckiana pervirens,* but split to 38 single and 13 double.

(d) The next day (September 19) one *pollicata* plant was found in family 33183, among the 113 offspring of a *Lamarckiana rubricalyx* old-gold single. All but one of these 113 plants were *Lamarckiana rubricalyx* old-gold single like the parent, the exception being a yellow-flowered plant which was probably introduced into this family

through some technical error. This yellow-flowered plant is omitted from the calculation of the linkage value for *pollicata*.

(e) On the same day (September 19) a plant of mut. *pollicata* was also found in family 33184 which was the progeny of a cross between two sibs, namely, *Lamarckiana rubricalyx* old-gold single and *Lamarckiana pervirens* old-gold double, the *pollicata* specimen being one of three *pervirens* plants in a family of 174, all the rest of which were *rubricalyx*, the number of *pervirens* plants having been reduced from otherwise expected equality with the *rubricalyx* group through the association of the *pervirens* factor with an egg-lethal (Shull, 1932).

The foregoing five families have, with respect to the specific topic of the present paper, nothing in common, except that the progenies have consisted of *Lamarckiana*-type plants, and that they have borne normal tubular hypanthia in all but the *pollicata* specimens above enumerated. Without reference to the other problems for whose solution these cultures were grown, these families may be totaled as consisting of 466 non-*pollicata* and 7 *pollicata*. Since the *pollicata* gene is a recessive, associated with the *Lamarckiana* zygote-lethals, each appearance of *pollicata* in these five families may be interpreted as the result of a crossing over whereby the *pollicata* gene has been transferred from the one complex to the other, that is, from a state of coupling with the one lethal, l_1, for example, to a condition of coupling with the other lethal, l_2, or *vice versa*. Assuming that this transfer or crossing-over from the one complex to the other occurred with like frequency in both megasporogenesis and microsporogenesis, this result would suggest, as a preliminary approximation, a crossover value of about 1.5 per cent.

In addition to these five *Lamarckiana* families which presented unexpected occurrences of mut. *pollicata*, three other families showed a condition of very exceptional interest in this connection. In each of these families the parent was a self-fertilized specimen of mut. *erythrina*

phenotype, a form characterized by a circle of six chromosomes and four pairs or by a circle of eight and three pairs, and behaving as a so-called "half-mutant" because it possesses only one of the zygote lethals of *Lamarckiana* and splits out in each generation a *decipiens* component, commonly approaching a 2:1 ratio, but often deviating quite widely from this ratio.

(f) The first of these three *erythrina* families discovered was family No. 33203, the progeny of a self-fertilized *pervirens erythrina*. This family split to 98 *erythrina* and 26 *decipiens*. Only 19 of the latter bloomed, every one a *decipiens pollicata*, while all the 98 *erythrina* plants had normal tubular hypanthia. This very definite association of the non-*pollicata* character with the zygote lethal of *erythrina* was a most striking demonstration of the inclusion of the *pollicata* gene in the first linkage group, and serves to further justify the assumption that the appearance of a *pollicata* plant in the progeny of *Lamarckiana* is to be interpreted as the transfer of a recessive gene for which the *Lamarckiana* parent was heterozygous, from one complex to the other.

(g) The second family derived from a selfed *erythrina* parent, and containing *pollicata*, is in several respects the most notable of all. This family (3351) was the offspring from a long ancestral line of selfings, beginning in 1919 with a typical *Lamarckiana* from the fourteenth generation of my cross-bred strain of that species. All the breeders in this line for twenty-five generations were typical *Lamarckiana*, but for the last four years the ancestral line has been repeatedly composed of *erythrina* plants, the first of these having been a mutant in *Lamarckiana* family 2930 grown in 1930. The family 3351 consisted of 86 *erythrina* non-*pollicata*, 75 *decipiens pollicata*, 1 unknown narrow-leafed mutant *pollicata* and 1 *erythrina pollicata*. The most interesting new feature of this family is the occurrence of one *erythrina pollicata* as a result of crossing over from the haplo-*decipiens* genome to the modified *velans* genome of *erythrina*.

(h) On September 19, family 33189 was also seen to consist of a mixture of *decipiens pollicata* and *erythrina*-like non-*pollicata*. All the *decipiens pollicata* plants which flowered (23 out of 31) were double-flowered *pollicata*, while 96 (out of 109) of the non-*decipiens* plants were double-flowered non-*pollicata*. The parent of this family had been classified in the rosette stage as *Lamarckiana*, and some of its offspring were also considered in the rosette stage as phenotypically equivalent to *Lamarckiana*, but microscopic examination of the parent showed the chromosomes arranged as a circle of six and four pairs, as in *erythrina*, and the production of a class of 36 *decipiens* segregates to 110 non-*decipiens* likewise accorded with the breeding behavior of *erythrina*.

Just as the five *Lamarckiana* families in which mut. *pollicata* was found were added together, so the three *erythrina*-like families may be summated as 286 *erythrina* non-*pollicata*, 117 *decipiens pollicata*, 1 narrow-leafed mutant *pollicata* and 1 *erythrina pollicata*. Only this last plant can be certainly designated as the result of a crossing over, by which the *pollicata* gene passed from a chromosome of the alethal haplo-*decipiens* complex to a corresponding chromosome in the lethal-bearing "modified *velans*" complex. This represents a crossing over of about 0.25 per cent. If the narrow-leafed mutant *pollicata* be assumed to be likewise due to a crossing over, this value would be doubled, namely, about 0.5 per cent.

Fig. 1 shows some of the variation in these several new examples of mut. *pollicata*, including four specimens from *Lamarckiana* phenotype, one from *erythrina* and two from *decipiens*. For comparison a normal *erythrina* non-*pollicata* is also included. The solid hypanthia of these *pollicata* flowers have been sliced down at the distal end whenever the hypanthium was partly tubular, and similarly the normal hypanthium has been sliced down to the ovary to expose the free style to where it connects with the top of the ovary. The most striking deviation is that shown in *erythrina pollicata* 3351 (113), where the hy-

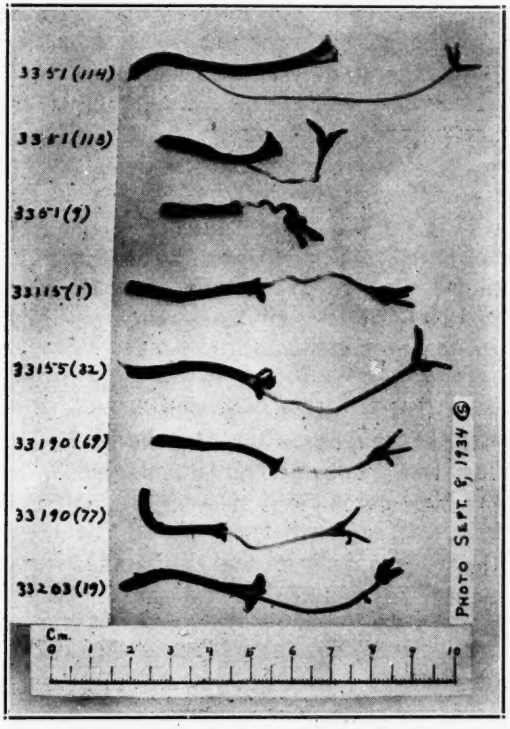

FIG. 1. Flowers taken from five of the eight families in which the unexpected occurrence of *pollicata* plants was observed during the summer of 1934. From these flowers the sepals, petals and stamens have been removed and the tubular portion of each hypanthium has been sliced down to the point of origin of the style. The "normal" non-*pollicata* condition is shown in 3351 (114).

panthium is shown, half solid, half tubular. This was an exceptional condition for this plant, as later flowers showed the hypanthia solid through nearly their entire length.

DISCUSSION

The appearance of these new examples of *Oe.* mut. *pollicata* in eight different families, none of whose known ancestors had solid hypanthia, suggests that the original

gene-mutation which has made such manifestations possible was not of as recent occurrence as I had supposed. In trying to understand just what has taken place, I have diagrammed the pedigree lines of the eight families in which these new mutants have appeared, together with the line which contained the original specimen 31303 (46) and its current offspring of family 33115. A study of this diagram (Fig. 2) shows several interesting facts. With the exception of family 3351, all these *pollicata*-containing families have come from a common pair of ancestors grown in 1928, and it seems reasonable to suppose that the *pollicata* gene was then present in at least one of these common ancestors. Whether it existed as far back as would be necessary to connect the ancestry of family 3351 (the top line in the diagram) with the other families, is an interesting but probably an unanswerable question. There has been no connection between this line and the other families since 1920 (14 generations) when ancestors of the two groups were both simultaneously included

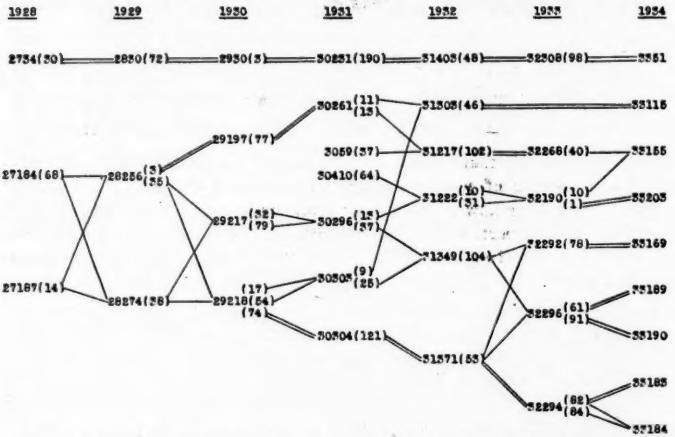

FIG. 2. Diagram of the pedigree connections of the families in which new and unexpected origins of *Oenothera* mut. *pollicata* were observed. Eight additional generations would be required to connect the line which leads to family 3351 with the other families. As this line was continued each year by selfing through a period of 14 generations nothing would be gained by diagramming the intervening families.

for the last time in my cultures of cross-bred *Lamarckiana*. The long line of repeated selfings which have been carried on in the line leading to 3351 makes it almost incomprehensible that the *pollicata* gene should have been present all that time and have failed to manifest its presence for so many years. I believe the more reasonable explanation is a repetition of the *pollicata* gene mutation in the two lines.

These new occurrences of mut. *pollicata* have made it possible to study the behavior of this gene in association with several other traits, but not yet in combination with mut. *brevistylis*. It appears now that mut. *pollicata* is not dominant or epistatic to *supplena* (double flowers), as stated in my first paper on this mutant type (Shull, 1934). In families which are segregating both double flowers and solid hypanthia, these two recessives are capable of coincident though somewhat modified expression, except in those forms of *supplena* in which there is complete suppression of the style, and the gynaecium becomes a small tuft of leaflike carpellary phyllomes growing out of the top of a wholly non-functional ovary. In this extreme type of *supplena* the tube of the hypanthium is very much reduced in length and the *pollicata* character is usually not observable. In many of my double-flowered strains the ovary and styles remain functional, though commonly more or less strongly modified by the presence of the *supplena* gene. In these strains, the *pollicata* gene changes the tubular hypanthium, at least to some extent, to a solid one, so that both *supplena* and *pollicata* traits are recognizable, although there is some antagonism between the expressions of these two genes, which renders the recognition of *pollicata* less easy in double-flowered than in single-flowered plants. Both *supplena* and *pollicata* genes commonly have the effect of shortening the hypanthium relative to the length of the bud-cones, and the heavy stigmas of *pollicata* usually modify the taper of the bud-cones. The usual position of the stigmas in the *pollicata* buds is in the midst of the group of opening anthers, as

described in my previous paper dealing with this form (Shull, 1934), but a few plants have been found in which the proximal portion of the styles has been mechanically stronger than usual, with the result that in these cases the stigmas are carried beyond the stamens far enough to prevent automatic self-fertilization. The condition previously described is very much the more common, however.

It is always of interest to carry the analysis of gene-action as far as possible in order to understand as clearly as may be what is the fundamental effect of the given gene. In the case of such a structural characteristic as the solid hypanthium produced in the duplex presence of the *pollicata* gene, it can usually be recognized that the normal coordination of cell divisions and of cell differentiations have been modified within certain definite regions. While it is customary to speak of gamopetalous corollas as formed of "fused" petals, the expression "fused" misinterprets the actual manner of production of such "compound" structures. In the primordium of a flower, there is approximately uniform longitudinal growth in all portions of the central axis up to a certain level, where the torus is located. At the distal end of this region of common growth, certain areas cease to grow, or become of relatively slower growth, while others continue to grow at a relatively greater speed of elongation. In this way the separate floral parts are delimited by lines or areas of inhibited or retarded growth. The so-called "fusion" of two adjacent structures results from the elimination of the inhibition or retardation of growth in the interval separating their primordia; thus instead of two or more separate enations, there is a single outgrowth from a larger common basal area. In the normal Oenothera flower, growth at a small central area at the end of the region of common growth produces the style. Surrounding this stylar enation is a narrow circular zone in which longitudinal growth has ceased at the distal end of the ovary. A second concentric zone continues to grow for-

ward, forming the hypanthium, from the distal end of which the four sepals, four petals and eight stamens develop. It is thus seen that a fundamental difference between the solid hypanthium of mut. *pollicata* and the hollow hypanthium of the normal Oenothera flower is caused by the change of a dominant gene which inhibits further longitudinal growth in a narrow circular zone surrounding the place of outgrowth of the style at the distal end of the ovary, to a corresponding recessive gene, which permits continued growth in this circumstylar zone.

This recognition that the normal gene inhibits growth in a particular structure, at a particular point in its development, and that the new recessive gene removes such inhibition, reminds one of the classic case of the long-tailed Japanese Tosa fowl, in which similarly a recessive gene has replaced the inhibiting effect of its normal dominant allele.

When I read my previous paper (Shull, 1934) on *Oenothera* mut. *pollicata,* at the Cambridge, Mass., meeting of the Botanical Society of America, Professor J. H. Schaffner pointed out that the modification of floral structure produced by the *pollicata* gene results in a type of floral structure not included among the eight floral types into which he had classified all the different kinds of angiospermous flowers (Schaffner, 1925). A further consideration of this question has led to the recognition of a few other cases in which there are normal conditions somewhat analogous to that produced by the *pollicata* gene, but no such case is known in any species closely related to Oenothera. The interpolation of a solid stem-like structure between the distal end of the ovary and the proximal end of the style, which is a wholly new structure in the Onagraceae, may be recognized, for example, as somewhat analogous to the formation of beaked achenes in certain Compositae, Cichoriaceae, etc. Even though the solid hypanthium intervening between the ovary and the style, which results from the presence of this new gene, can not be considered absolutely unique, as it was at first

thought to be, the effect of this new gene is such as would be needed to introduce into any evolutionary sequence a wholly new characteristic. This fact has considerable significance in relation to the question whether gene mutations may have resulted in important deep-seated differences in the organization of plants and animals, or whether they relate necessarily to relatively insignificant superficial differences. That such wholly new departures may be introduced by gene mutations into the evolutionary sequences of plants and animals must be assumed if genera, families and higher classificatory categories are to be understood as being genetically related to one another in the sense that they have had common origins.

The crossover ratios observed in the families dealt with in this paper, 1.5 and 0.25 per cent., can be considered only preliminary suggestions and may deviate considerably from the ratios which will be derived from a study of a larger series of backcross families. The data are still too meager to permit a decision as to whether there is a real difference in frequency of transfer of the gene of mut. *pollicata* from one complex to the other, when the two complexes involved are *gaudens* and *velans* than when they are haplo-*decipiens* and "modified *velans*." It seems reasonable to suppose that such differences may exist in the frequency of transfer between the complexes, when different pairs of complexes are involved. The whole question of linkage behavior in the Oenotheras needs a much greater amount of intensive investigation than has yet been made, and the discovery of new genes, such as mut. *pollicata*, provides the necessary material for the making of such analyses.

SUMMARY

(1) *Oenothera* mut. *pollicata*, which is distinguished by a solid hypanthium intervening between the distal end of the ovary and the proximal end of the style, recurred unexpectedly in 1934 in eight different families. Five of these families were from *Lamarckiana*-like parents and three from *erythrina*-like parents.

(2) The distribution with respect to other segregating traits showed that mut. *pollicata* is independent of genes of the third linkage group and closely linked with genes of the first linkage group.

(3) The presence of the well-known zygote lethals in the first linkage group conditions the manifestation of *pollicata* just as it does that of other first-linkage-group recessives. In progenies from selfed heterozygotes of *Lamarckiana* type, *pollicata* can appear only as the result of a transfer from the *gaudens* to the *velans* complex or *vice versa*. In the five progenies here reported from *Lamarckiana* parents, there were 466 non-*pollicata* and 7 *pollicata*, indicating a frequency of crossing over of the general order of 1.5 per cent.

(4) In three families from *erythrina*-like parents, there was the usual segregation of *erythrina* and *decipiens*, in which all *decipiens* plants were *pollicata*, and all but one of the *erythrina*-like segregates had normal tubular hypanthia. In these three families there was a total of 403 plants, only one of which could be recognized certainly as a crossover, thus suggesting a crossover ratio of the general order of 0.25 per cent. (or of 0.5 per cent. if a narrow-leafed *pollicata* mutant be also assumed to have resulted from crossing over).

(5) The occurrence of these new examples of mut. *pollicata* is supposed to be due to the unsuspected presence of the *pollicata* gene, generally, in those families which were derived from a common pair of ancestors in the cultures of 1928, but it is more difficult to believe they could have escaped detection through the fourteen generations required to connect all these families. It is deemed more probable that the *pollicata* gene-mutation has been repeated.

(6) The *pollicata* gene removes an inhibition of growth at the top of the ovary in a narrow concentric ring separating the proximal portion of the style from the proximal portion of the hypanthium. It also diminishes the longitudinal growth of the hypanthium relative to that of

ovary and bud-cone, and modifies the differentiation of mechanical tissue in the style.

(7) The interpolation of a solid stem-like structure between the distal end of the ovary and the proximal end of the style as the result of a gene mutation makes easier an understanding of the manner in which different genera, families, orders, etc., may have originated.

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SHORTER ARTICLES AND DISCUSSION

DOES THE QUANTITY OF CHROMATIN PRODUCE A GENETIC EFFECT?

In a recent review on auto- and allopolyploidy Müntzing (1936) summarized our knowledge of these phenomena. If we are considering here only the physiological side of the case there stands out one general result: In the overwhelming majority of cases, including experimental as well as natural polyploids and including also the few cases in the animal kingdom (Artemia, Solenobia), there is a correlation between chromosome number and certain physiological and ecological properties. Most conspicuous among these is the alteration in growth rate, which, as a rule, becomes slower with the increase in chromosome number. It is assumed (with Wettstein, 1924) that this and other physiological peculiarities of the polyploids in regard to assimilation-energy, winter-hardiness, osmotic conditions are ultimately the outcome of the changed cell-size, which is within the limits of a certain formula (Wettstein, 1924) proportional to the number of chromosomes.

If these facts are viewed from the standpoint of a geneticist who is interested in the problem of the action of the hereditary material in controlling characters, an interesting question appears. Cell-size, within a species, is, according to Boveri's rule, proportional to the number of chromosomes within the nucleus. This might mean that the total mass of chromatic material determines nuclear and therefore cell-size. In this case the quality of the chromosome material, *i.e.*, the genes contained in the chromosomes, would be irrelevant. The effect on cell-size would be the same, for example, if the chromosomes A B C or otherwise D E F would be present in triplicates, provided they had the same size. The other alternative would be that not the quantity of chromatic material would be responsible for the phenomenon but the increase in number of definite genes controlling intracellular metabolism. If this be true we should deal with a special feature of ordinary genetic conceptions. If, however, the former alternative would prove to be true, this would amount to a genetic effect of chromatic material of the chromosomes independent of the genes. Theoretically, this alternative could be de-

cided by measurement of cells from different types of hyperploids of the same species. But here the differences in cell size would be hardly sufficiently large to allow a decision.

But there is another possibility of approach to this problem. In our work on the genetics of geographic variation in *Lymantria dispar* we found that all races of this moth have the same chromosome number n = 31 but that different races have chromosomes of different size and therefore a different quantity of chromatin, though there is no reason to believe that they possess different numbers of genes (see, however, the end of this paper). The details of the cases are to be found in Goldschmidt (1932). These facts provide a chance to attack the question which we put above. One of the typical consequences of polyploidy was a change of rate of growth and incidentally of the life-cycle (biennial, perennial, etc.). In Lymantria we have now a series of races characterized by different chromatin-quantities in which simultaneously typical hereditary differences in regard to growth-rate, life-cycle, cell-size have been studied. If the quantity of chromatin has anything to do with these differences the following two results are expected: (1) After crossing races with large and small chromosomes in F_2 a segregation must occur of the same type as if 31 pairs of polymeric genes were involved in the cross, which would mean practically intermediate inheritance in experimentation with usual numbers; (2) the hereditary characters supposed to be caused by the chromatin quantity must show within a set of different races a positive correlation with the respective condition of the chromosomes.

The first point to be considered is the relation of cell size to chromatin quantity. A very large body of measurements was taken for the size of spermatocytes and wing scales in a considerable number of races. The details are recorded in Goldschmidt (1932). These were compared with the data obtained for chromatin quantity in these races. No correlation was found; cell-size was not dependent upon chromosome size. Moreover, it was found that in this case cell-size was positively correlated to body-size. Large races had also large cells and *vice versa*. Of course then body-size was neither correlated with chromatin-quantity.

The next character to be accounted for is the growth rate, which is known to be profoundly affected by polyploidy. Growth rate is typically different genetically in different geographic races of Lymantria (with additional sexual differences which show also

independent genetic variation). Growth rate was, however, found to be genetically independent of the velocity of differentiation, which could be proven to be a genetic property adapting the life cycle to the seasonal cycle. In a general way the differences in body-size and growth rate showed the same rules as found by Castle and Gregory for rabbits: the eggs of different races were of identic size, but the larger races had from the start a higher growth-rate. (Details in Goldschmidt, 1933.) This, however, did not mean that the larger races had more cells of the same size as those of the smaller races: we mentioned already the positive correlation between body size and cell size. A comparison of growth-rate and body size as well as of velocity of differentiation in the different races with the respective chromatin quantities, however, reveals no correlation. In addition the mode of inheritance of these characters (see Goldschmidt, 1933) is not of the type which would be expected if differences in chromatin were involved.

Finally also the features of the life-cycle of different races could be tested. One of the typical racial characters of Lymantria is the length of the diapause *ceteris paribus*. This again is one of the genetically determined racial differences which could be proven to be of an adaptational type. (For details see Goldschmidt, 1932a). Again this physiological property, which in plants seems to be influenced by polyploidy, proved to be not correlated in the different races to the quantity of chromatin. All these facts then show clearly that different quantities of chromatin within the same number of chromosomes do not have a similar effect as different chromatin-quantities produced by doubling, tripling, etc., of some or all chromosomes. From this the conclusion might be derived that it is not the chromatin quantity, as, *e.g.*, Wettstein is inclined to assume, which accounts for the typical polyploid effects but the actual number of the chromosomes. And whereas in our case also the surface of the chromosomes is ruled out as active agent, the conclusion seems warranted that the decisive factor is not the quantity of chromatin but the quantity of some or all genes present, whatever this might mean in terms of gene action.

On the basis of our results it is, however, not permitted to draw the further conclusion that the quantity of chromatin (without change of chromosome number) is without genetic significance. In working out the correlations between chromatin quantity and the genetic characters of the different geographic races an unex-

pected positive correlation was found, namely, with the different "strong and weak" sex-races: all weak races had large chromosomes, strong races small chromosomes and "neutral" races intermediate chromosomes. (For details and pictures see Goldschmidt, 1932.) But these sex-races are characterized by the condition of both female and male determiners. The latter ones (M) are situated within the X-chromosomes, but the former ones (F) are proven to be inherited with the cytoplasm, *i.e.*, maternally, with the Y-chromosome excluded (see Goldschmidt, 1934). In strong and weak races both F and M are strong and weak, respectively, in neutral races F is pretty strong and M neither weak nor strong. M is inherited like a single sex-linked gene. But what is F within the cytoplasm? In Goldschmidt (1934) the possibility has been discussed that F is a cytoplasmic property controlling a threshold for the action of M. The question now arises: Is it possible that the relative strength of this cytoplasmic property F is in some way controlled by the mass of chromatin, as suggested by the established correlation? The answer is negative: otherwise reciprocal crosses weak × strong and strong × weak ought to give identic results, if not in F_1 at least in F_2, which is not the case. Thus only two possibilities are left: Either the cytoplasmic condition called strong and weak F controls the size of the chromosomes, which would be a parallel to Boveri's classic case of cytoplasmic control of chromatin-diminution in Ascaris; or the established correlation sex race-chromosome size is a chance result without significance. In any case no genetic effect of the quantity of chromatin can be demonstrated.

Finally, the possibility ought to be considered that the strange parallelism: strength of sex-determiners—chromosome size is a consequence of internal duplications of chromosome material, visible as an increase in chromosome size. This would put our quantitative explanation of strength on a very interesting basis. Thus far no attack upon this problem is visible, as Lymantria has no giant salivary chromosomes and no mapped genes. If such an explanation were applied, however, the question of chromatin quantity versus gene differences would cease to exist. In view of the recent work of Dobzhansky and others on differences of chromosome architecture connected with geographic variation, the point of view just mentioned might become important in the future.

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MODIFICATIONS OF THE COMPOUND EYE OF *DROSOPHILA MELANOGASTER* ARISING UNDER X-IRRADIATION[1]

In the course of a series of investigations the ultimate object of which is a statistical evaluation of the volume of the locus for the genic character "white" in the fruit fly *Drosophila melanogaster,* a considerable number of modifications of form and color of the compound eye under x-irradiation have been observed, which are considered worthy of record at the present time, in the hope that they may be of interest to other workers in the field.

The procedure used involved the examination of a very large number of imagines of homozygous eosin stock, obtained from Turtox Service, which had been subjected as eggs and young larvae to x-rays from a Coolidge tube with tungsten target, operated at 85 kvp. and 10 ma. at a focal distance of five inches, the tube being equipped with a thin sodium-glass window and the radiation otherwise unfiltered. The output of the tube has been determined at 78.1 r/min. with a Victoreen dosimeter, and times of exposure in the present experiments ranged from one half to four minutes. This work essentially represents a continuation of that of Patterson (1929) and several of the modifications observed have already been described by him. No interpretation of any modifications is attempted at present. The following changes were observed.

(1) *Mosaic compound eyes in males.* This is an effect reported by Patterson, and histologically examined by Casteel. Mosaic patches of ommatidia on the compound eye are white, the background being typical light (male) eosin (Fig. 1).

[1] From the Biological Laboratories, Harvard University, Cambridge, Massachusetts, and the Haskins Laboratory, Schenectady, New York.

(2) *Mosaic compound eyes in females.* Also reported by Patterson and examined by Casteel. The background is of dark (homozygous or female) eosin, the mosaic patches of light (heterozygous or male) eosin (Fig. 2).

(3) *White mosaic compound eyes in females.* The background is of dark or homozygous eosin, the mosaic white as in the male. Much rarer than the preceding case. Work designed to aid in its interpretation (possibly as the simultaneous inactivation of both loci) is in progress (Fig. 3). Areas of modified coloration of all types may be multiple, several occurring in a single eye (Fig. 4).

(4) *Irregular rows of ommatidia.* The normal arrangement of facets in the eye is disturbed by irregular rows and patches of disordered facets, sometimes scarcely tangent to those adjacent. This modification is very frequent in x-rayed stock, and it occurs in a certain percentage of control material (Fig. 5). A condition of duplicated facets is often associated with this modification.

(5) *Missing lenses.* Very rarely a condition has been found in which patches of otherwise perfectly normal ommatidia completely lack the distal lens (Fig. 6).

(6) *Pits.* In circumscribed areas facets have been found completely lacking in certain individuals, with the resultant formation of craterlike pits in the eye surface (Fig. 7).

(7) *Swollen areas.* Areas similar in form to the preceding are composed of facets apparently of considerably greater length than normal, resulting in the production of an elevation above the general eye surface (Fig. 8).

(8) *Sunken areas.* Whole groups of otherwise normal facets appear unusually short, resulting in markedly depressed regions of the compound eye. The effect is very similar to that often appearing in material which has been dead for several hours, and in which the eyes have partially shrivelled (Fig. 10).

(9) *Scars.* The edge of the eye appears notched, as though ommatidia were missing at the margin. Often the scar formation is accompanied by several whitened facets (Fig. 11). The rim of the eye may also be discolored, brown spotting sometimes appearing, but more frequently a whitening which may extend to the thorax.

(10) *Double lobed eye.* The compound eye shows a tendency to separate into two lobes (Fig. 10).

(11) *Miniature eye.* The number of ommatidia is considerably reduced, often nearly to half of the normal. The modifica-

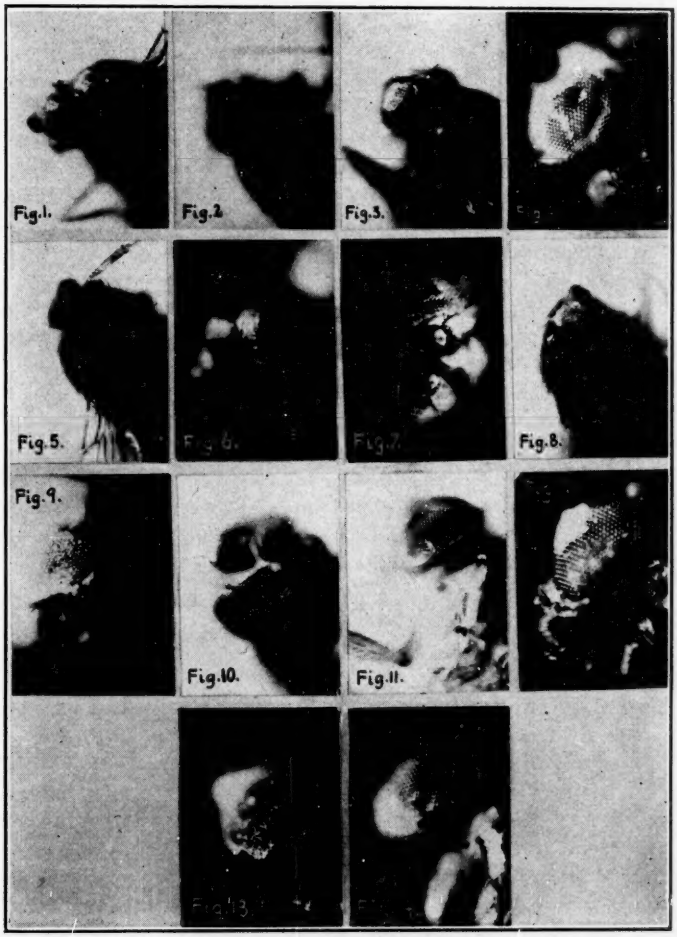
Fig.1.
Fig.2.
Fig.3.
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Fig.10.
Fig.11.

tion has been seen only twice in a count of 32,000 individuals, once in irradiated and once in control stock. In the former case one eye was miniature, the other normal; in the latter, both were miniature.

(12) *Deformed setae.* Deformed setae are usually thick and club shaped, rarely hooklike (Fig. 9). In a very few cases, setae have been found missing over the area of the eye facets.

(13) *Tumors.* These striking modifications are sometimes found associated with white mosaic patches in females. They present unusual shapes, as dome-like elevations, rounded lobes overhanging the rim of the eye or horn-shaped protrusions. Scattered ommatidia occasionally occur on them, especially about the margins. These tumors are usually white, rarely pigmented (Fig. 12).

(14) *Compound effects.* Often several of the above-described modifications occur in a single eye. Such are combinations of tumors and areas of white facets, white facets and pits, tumors, pits, white facets and irregular arrangements of rows of ommatidia, and white facets and deformed setae (Figs. 12 and 13).

(15) *Reverse color variations.* These, of very considerable interest, have been described elsewhere.[2] They comprise reverse mutations from white eye to wild type and cases of mutation from eosin to a darker intermediate shade of eye color, probably vermilion.

SUMMARY

A number of modifications of eye color and form which have been observed in homozygous eosin stock of *Drosophila melanogaster* are figured and described. These were found in the course of an investigation of the production of mosaic patches of whitened or lightened ommatidia in imagoes arising from eggs and young larvae subjected to x-rays, involving a very large number of individuals and undertaken in the course of a statistical evaluation of the volume of the white locus. The majority of the modifications were observed in x-rayed stock, but a few were also found in lower frequency in control material. Several have previously been described by Patterson. No interpretation of any of them is attempted at present.

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[2] Enzmann and Haskins (in press).

DEVELOPMENT OF EYE COLORS IN *DROSOPHILA*: THE MUTANTS BRIGHT AND MAHOGANY

In a general survey of the development of pigment in eye transplants in *Drosophila melanogaster* (Beadle and Ephrussi, 1936) evidence was obtained for the existence of three separate but related diffusible substances. The tentative names and identifying characteristics of these may be given as follows:

(1) *ca*$^+$ *substance*—a substance necessary for the development of wild type pigment which is absent or reduced in amount in the eye color mutant claret.

(2) *v*$^+$ *substance*—a substance capable of changing the development of a vermilion eye in such a way that it forms pigment phenotypically like that of a wild type eye.

(3) *cn*$^+$ *substance*—a substance capable of changing the development of a cinnabar eye in such a way that it is similar in its pigmentation to a wild type eye.

Of the known eye color mutants, claret alone has been found to be deficient in *ca*$^+$ substance. Vermillion flies lack (or have reduced amounts of) both *v*$^+$ and *cn*$^+$ substances. Several other eye color mutants have reduced amounts of these two substances. (Additional tests indicate that the original conclusion, Beadle and Ephrussi, 1936, that carmine, peach and ruby lack both *v*$^+$ and *cn*$^+$ substances is incorrect; they are characterized by reduced amounts of these substances as compared with wild type but not by their total absence.) Cinnabar was the only mutant found to lack only *cn*$^+$ substance. In all other mutants studied it was found that if *v*$^+$ substance was present in reduced amount, *cn*$^+$ substance was likewise found in reduced amount.

It is the purpose of this paper to present data from transplantation experiments involving two recently discovered eye color mutants. These are of interest because, when used as hosts, both have sufficient *v*$^+$ substance to change vermillion to wild type, but only enough *cn*$^+$ substance to cause a partial change in cinnabar toward wild type.

The Mutant Bright (*bri*)

The eye color mutant bright was found in a stock homozygous for small eye (*sy*) by Mrs. E. N. Skoog of this laboratory. The eye color of *bri* flies resembles cinnabar-2 in being somewhat brighter than wild type. Separation of *bri* from wild type is often quite difficult. The locus of *bri* was found by Mrs. Skoog (unpublished) to be close to that of Bristle in chromosome 2,

probably about 0.5 units to the left. Since the indications were that the locus of *bri* is relatively close to that of the more or less similar mutant *cn*, tests were made by Mrs. Skoog to determine whether the two mutants were allelomorphic. F_1 flies from the cross *cn* x *bri* were found to be phenotypically wild type. In the course of the work reported here, similar tests were made with the same result. Thus the direct tests indicate that *cn* and *bri* are not allelomorphic.

The results of eye transplants involving bright are shown in Table 1. Unfortunately the difference between *bri* and wild type

TABLE 1*

DATA FROM TRANSPLANTATION EXPERIMENTS INVOLVING THE EYE COLOR MUTANT BRIGHT

Implant	Host	Number of individuals	Phenotype of implant
bri	+	3, 1, 6, 1 ; 11	+ (?)
+	*bri*	1, 2, 5, 7 ; 15	+ (?)
cn	*bri*	10, 8, 6, 5 ; 29	*cn* (slightly darker)
w^a *cn*	*bri*	2, 2, 4, 3 ; 11	darker than *w*^{a} *cn*
v	*bri*	11, 5, 10, 7 ; 33	+
cn	*cn/bri*	6, 6, 7, 5 ; 24	+
v	*cn/bri*	7, 4, 4, 4 ; 19	+
cn	*bri/+*	1, 0, 0, 3 ;4	+

* In this and the following tables, under the heading "number of individuals," are given the four sex combinations in the order : female in female, female in male, male in female, male in male, and total.

eyes is so slight that the results of growing *bri* eye disks in wild type hosts and the reciprocal are not clear. The indications are that *bri* implants grown in wild type hosts have wild type pigmentation and that a wild type implant in a *bri* host likewise has wild type pigmentation.

Three experiments made at different times agree in showing that a *cn* implant grown in a *bri* host is phenotypically close to *cn* in *cn* control plants. There appears to be a relatively slight modification in the direction of wild type. Tests made by implanting *w*^{a} *cn* eye disks, known to be more sensitive detectors of small amounts of *cn*$^+$ substance than *cn* implants (Ephrussi and Beadle, in press), show a distinct modification in the pigmentation of the implant in the direction of *w*^{a}. These results indicate that a *bri* host is capable of supplying an implant with but a small amount of *cn*$^+$ substance as compared with that supplied by a wild type host.

Three experiments in which *v* implants were grown in *bri* hosts show that a *bri* host can supply a *v* implant with sufficient *v*$^+$ substance to result in the formation of wild type pigmentation.

The above results show that in these experiments the mutant *bri* behaves in essentially the same way as does *cn*. One is led to wonder whether, in spite of the direct test, *cn* and *bri* are not actually allelomorphic. The transplantation experiments suggest another way of testing for allelomorphism. The mutant *cn* lacks cn^+ substance and *bri* has only a small amount. If the two mutants are allelomorphic, one should expect flies of the constitution *cn/bri* to develop only a small amount of this substance. The results of growing a *cn* implant in a *cn/bri* host show that this is not the case; such implants are phenotypically very close to wild type.

A *cn* implant is known to result in the modification of the normal eyes of a w^a *v* host. Tests for such an effect, using one and two *bri* implants (Table 2), were negative. In this respect, then,

TABLE 2

DATA ON THE EFFECT OF BRIGHT OR HETEROZYGOUS BRIGHT IMPLANTS ON THE EYE COLOR OF w^a *v* AND w^a *cn* HOSTS

Implants	Host	Number of implants developed	Number of individuals	Phenotype of	
				Implant	Host
bri	w^a *v*	1	1, 0, 0, 0; 1	*bri* (?)	w^a *v*
bri	w^a *v*	2	3, 2, 4, 2; 11	*bri* (?)	w^a *v*
bri	w^a *cn*	1	2, 3, 0, 2; 7	*bri* (?)	darker than w^a *cn* (?)
bri	w^a *cn*	2	0, 1, 0, 1; 2	*bri* (?)	darker than w^a *cn* (?)
cn/bri	w^a *v*	1	1, 0, 0, 1; 2	+	w^a *v*
cn/bri	w^a *v*	2	3, 2, 4, 0; 9	+	w^a *v*
cn/bri	w^a *cn*	1	0, 3, 0, 2; 5	+	darker than w^a *cn*
cn/bri	w^a *cn*	2	1, 3, 2, 3; 9	+	darker than w^a *cn*

bri differs from *cn;* both produce v^+ substance as determined by using them as hosts, but *cn* releases this substance from an implanted eye, while *bri* does not. An implanted eye of the constitution *cn/bri* behaves in the same way as does a wild type implant in that such an implant does not modify the normal eyes of a w^a *v* host, but does modify the color of the normal eyes of a w^a *cn* host (toward w^a—see Ephrussi and Beadle, in press). There appears to be a very slight modification of the eyes of a w^a *cn* host by a *bri* implant, a fact consistent with the experiments mentioned above in indicating that in the mutant *bri,* a small amount of cn^+ substance is produced.

THE MUTANT MAHOGANY (*mah*)

The eye color mutant mahogany (not to be confused with the mahogany—*my,* 3–49.5±—eye color mutant described by Bridges and Morgan, 1923, which has been lost) was found in homozygous

form in the *cn* stock in Pasadena and also in a *cn* stock obtained from Professor R. Goldschmidt. The eye color in mahogany flies is clot-like, but less strikingly different from wild type. Separation from wild type is reliable but slow in young flies and more difficult in older flies. The locus of *mah* was determined from the cross *H Mw/mah* ♀♀ x *mah* ♂♂ to be 7.5 units to the right of *Mw* in chromosome 3 (that is, at about 87.5).

Data from transplantation experiments involving *mah* are summarized in Table 3. As in the case of *bri*, *mah* is so close to

TABLE 3

DATA FROM TRANSPLANTATION EXPERIMENTS INVOLVING THE EYE COLOR MAHOGANY

Implant	Host	Number of individuals	Phenotype of implant
mah	+	0, 1, 2, 1 ; 4	? (see text)
+	*mah*	1, 1, 0, 0 ; 2	+ (?)
cn	*mah*	3, 1, 2, 2 ; 8	Intermediate between *cn* and wild type
v	*mah*	2, 2, 2, 3 ; 9	+

wild type phenotypically that the results of implanting *mah* eye disks into wild type hosts are not clear. Of the four implants obtained of this type, two appeared similar to wild type control implants and two appeared to be similar to *mah* controls. Implants of wild type disks grown in *mah* hosts are probably wild type, but this is not certain. The transplantation of *v* disks to *mah* hosts gives implants with wild type pigmentation, showing that a *mah* host can supply v^+ substance to an implant. Eye disks from *cn* donors implanted in *mah* hosts develop pigmentation intermediate in appearance between *cn* and wild type. This indicates that, compared with a wild type host, *mah* supplies an implant with a reduced amount of cn^+ substance.

The results of other transplantation experiments involving the mutant *mah* are considered in other papers.

SUMMARY

The eye color mutants bright (*bri*, 2–54 ±) and mahogany (*mah*, 3–87 ±) are characterized by a reduced amount of cn^+ substance as compared with wild type, *i.e.*, when used as hosts to cinnabar eye implants, the pigmentation of the implant is modified in the direction of wild type, but in neither case is the modification complete. Both of these mutants as hosts to ver-

million eye implants result in a modification of the implants of such a nature that their pigmentation is like that of wild type, *i.e.*, the bright and mahogany mutants show no evidence of having less v^+ substance than a wild type fly has. The reactions of the bright and mahogany mutants to vermillion and cinnabar implants are different from those of the eye color mutants previously studied.

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THE "MYTH" OF THE LEMUR'S COMB

I THINK it would be a pity if the observations made by Cuvier and St. Hilaire (and by many other zoologists in the intervening century) as to the hair-comb function of the lower teeth of the lemurs should be lightly swept aside because Dr. M. Russell Stein[1] has been unable to confirm them by watching seven lemurs at the Bronx Park Zoo. There are many people who are very familiar with domestic poultry and who have watched them daily for years; and yet quite possibly the great majority of them have never seen a hen lay an egg. This fact would, however, not furnish sufficient grounds for a scientific man to refer to the egg-laying habit of the domestic hen as a "myth."

But on the anatomical side of the question observation is open to all. Dr. Stein says that "it requires considerable pressure to pass the finest waxed thread between these teeth. The contact points are tight. This holds true for all lemurs, alive, dead and skeletal preparations."

The first figure (reproduced from a photograph) in his paper, although not a very satisfactory illustration, clearly shows a different state of affairs from that described; for the spacing be-

[1] AM. NAT., 70: 19–28, January–February, 1936.

tween the teeth is very evident. There is enclosed a photograph from an untouched negative of a normal mandible of a specimen of *Lemur fulvus,* which died in the gardens of the Zoological Society of London twenty years ago. The condition of the procumbent front teeth is readily seen to be such as to admit the fine hairs of the lemur's fur to pass between the adjacent teeth.

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THE tonsorial functions of the lemur may be investigated from two angles, the habits of the live specimen and the skeletal preparation. In the study of the live specimen one must depend upon the subjective testimony of the observer, which, it must be granted, is subject to the influence of personal interpretation. There should be no controversy, however, over the anatomical form of the skeletal preparation.

I have just referred back to the specimens of *Lemur fulvus* at the American Museum of Natural History. They all have tight contacts at the tips of their anterior teeth.[1] To the best of my knowledge all lemurs show this same characteristic. I must frankly admit that I have never seen a normal lemur like the one submitted by Professor Wood-Jones. I have seen very similar specimens which had been damaged by over-handling and lying around a laboratory for several decades. The fine edges of enamel had chipped off and left spaces between the teeth.

This may not have been the case with Professor Wood-Jones's London lemur. His may have suffered from dental troubles during his earthly existence. The teeth may have broken down in old age or perhaps they are peg-shaped incisors like the human peg-lateral. Whatever the case may be, this specimen is not normal. The irregularity of the shapes and lengths of the teeth present a marked contrast to the fine symmetry and contour of those specimens collected from the natural habitat where the alleged combing mechanism is supposed to have evolved.

M. RUSSELL STEIN

[1] I have mailed photographs of this feature to Professor Wood-Jones.